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THE CENTENARY OF THE CHILDREN'S HOSPITAL IN GOTHENBURG, SWEDEN

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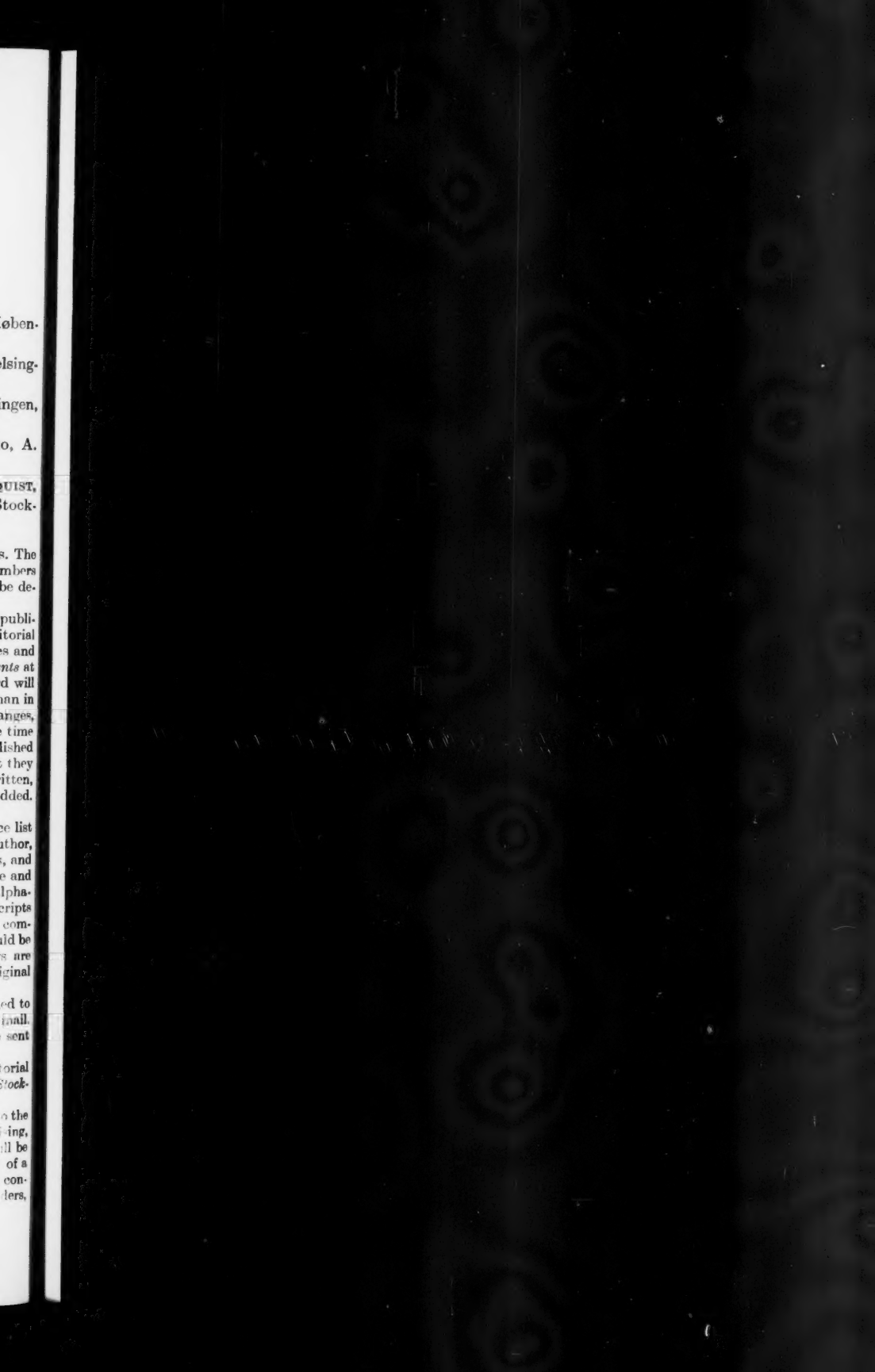

THE CENTENARY OF THE CHILDREN'S HOSPITAL IN GOTHENBURG, SWEDEN

Papers from members of the staff of its
Pediatric Department

Almqvist & Wiksells
BOKTRYCKERI AKTIEBOLAG
UPPSALA 1960

The Centenary of the Children's Hospital in Gothenburg

Quite recently the Children's Hospital in Gothenburg has celebrated its centenary.

The initiative in founding a children's hospital in Gothenburg was taken by a few far-sighted Gothenburg men. By generous grants they financed not only the building of a hospital but also its running for almost 40 years as a charity hospital without any support from the community.

In 1856 a wealthy merchant, Mr. D. Carnegie, who had lost a beloved son from a children's disease, pointed out the necessity of founding a hospital devoted solely to the treatment of sick children. In a letter to the Committee of the City General Hospital he expressed these views and also related how impressed he had been from reading the report on the activity at the Crown Princess Lovisa's Hospital in Stockholm opened two years earlier as the first children's hospital in Sweden. Through his great enthusiasm Mr. Carnegie was able to inspire other people for his ideas. In the organizing committee which was founded Dr. C. Dickson took a very active part.

The project progressed rapidly and in 1859 on November 24th, the Children's Hospital was opened in provisional premises. It was the second hospital of this type in Sweden but still one of the first in Europe. A physician particularly interested in pediatrics, Dr. G. Billqvist, was appointed to be responsible for the medical care. At the beginning the hospital had only 12 beds, but the number of outpatient visits was very high. After a few years it became clear that more beds were needed and already within another 6 years a new and better equipped hospital was built. The number of beds was gradually increased to 40. That still a larger hospital was necessary became evident in the beginning of this century. During the time when the Children's Hospital had been in activity it had been very much appreciated. As a result, the efforts which were made by Dr. Billqvist and his successor, Dr. G. Bergendal, to increase the funds of the hospital from private donations were successful and it became possible to move the hospital to a larger and more modern building.

In 1909, the hospital, which at that time had 134 beds, was opened at the same place were it is still situated. Apart from being enlarged in 1921 so that it now has about 260 beds, it has largerly remained in its original form.

In most ways the hospital is now organized as a complete children's hospital. As early as in 1912 it was divided into one medical and one surgical department. Since 1953 it has also had a department of child psychiatry. Various diagnostic departments have also been instituted. In addition, the hospital has one ward for prematures and sick newborns in each of the two maternity hospitals of the city, one convalescent home, one unit for children with cerebral palsy and another unit for mentally retarded children.

In 1922, when Dr. A. Wallgren was appointed as head of the medical department, an era of intense scientific research started at the Children's Hospital. It very soon attained an international reputation and was visited by pediatricians from all over the world. Until 1942, when Dr. Wallgren left the hospital to take over the Chair of Pediatrics at the Karolinska Institut in Stockholm, new contributions to various fields of pediatrics were reported by him and his assistants. Among the best-known of these are the introduction of a new method of BCG vaccination, the follow-up studies on the value of BCG, studies on the connection between erythema nodosum and primary tuberculosis, the studies on aseptic meningitis, reticuloendothelial granuloma, and on pyloric stenosis.

A milestone for the Children's Hospital was the foundation of a new medical school in Gothenburg in 1949. In 1954, Dr. Y. Åkerrén, who had succeeded Professor Wallgren as head of the medical department, was appointed as the first Professor of Pediatrics at the University of Gothenburg. Due to this untimely death he held that position only until 1957. Professor Åkerrén's scientific work was mostly devoted to newborn problems. Valuable contributions were made in the field of treatment of asphyxiated newborns. Another well-known study by him was the demonstration that cretinism may be a cause of prolonged neonatal jaundice.

Since the Children's Hospital became a teaching hospital its staff has expanded

markedly and the facilities for scientific work have improved.

In this issue of *Acta Pædiatrica* a series of papers from the Children's Hospital will be presented, to give an idea of present activities in its Pediatric Department.

ROLF ZETTERSTRÖM
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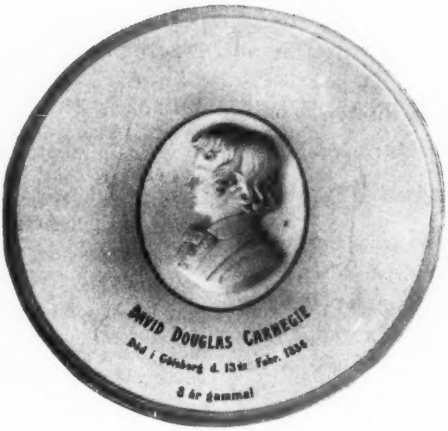

Acta Pædiatrica 49: 470—479. July 1960

From the Department of Paediatrics, Children's Hospital, University of Gothenburg, Gothenburg, Sweden

Juvenile Nephronophthisis. Part I

A Genetically Determined Nephropathy with Hypotonic Polyuria and Azotaemia

by O. BROBERGER, J. WINBERG and R. ZETTERSTRÖM

Introduction

The first report of an earlier unrecognized renal disease—"familial juvenile nephronophthisis"—was given in 1951 by Fanconi, Hanhart, von Albertini, Uhlinger, Dolivo & Prader (8). The report was concerned with two unrelated families with altogether ten children, seven of which were affected by the disease. Among these there were six boys and one girl. The familial accumulation and male preponderance was the most conspicuous etiological characteristic of the new disease entity. Typical clinical features were hypotonic polyuria with azotaemia in the absence of arterial hypertension, erythrocyturia and more prominent albuminuria. In one family all patients died before the age of six years. The histological findings seemed to be unique for this disease. Seven further cases have later been added, two by Grüttner & Lenz (10), four by Hackzell & Lundmark (11), and one by Hooft, Roels & Herpol (14).

The present communication is based upon a study of "juvenile nephronophthisis" in seven patients from six different families; it is an attempt to define the clinical and biochemical characteristics of this disease and to discuss the pathogenesis of the symptoms. Histological examination was performed in two patients and found to be similar to those described earlier. The interpretation of the histological changes and the pathogenesis of the symptoms is, however, different from the earlier ones. The autopsy findings will be reported in a separate paper of this issue (15).

Material and Methods

The material consisted of six girls and one boy. The age at the first admission was 6–16 years. Clinical and other data are presented in Tables 1–7 and Figures 1–5. Five patients have died. Post-mortem examination was performed on two but due to the wishes of the parents the autopsies were only partial. The other three patients died at home.

Alkaline phosphatase activity in blood serum was determined according to Buch & Buch (3). With this method the upper normal limit before puberty is about 16 units per ml serum and during puberty up to 30. Endogenous creatinine in plasma and urine was determined after extraction with Lloyd's

TABLE 1. *Reason for admission and admitting complaint.*

Case	
1	*Albuminuria.* Detected at routine school examination. No complaints.
2	*Anaemia.* Detected at routine check up at school after an upper respiratory tract infection. No complaints.
3	*Anaemia.* Tired, pale. Persistent anaemia detected at school examination.
4	*Elevated E.S.R.* Persistent elevation of E.S.R. during several years. Originally detected by chance. No complaints.
5	*Tetany.* Pains and intermittent stiffness of the legs.
6	*Family history.* Examined because of positive family history (Case 5). No complaints.
7	*Albuminuria.* Detected at routine school examination. No complaints.

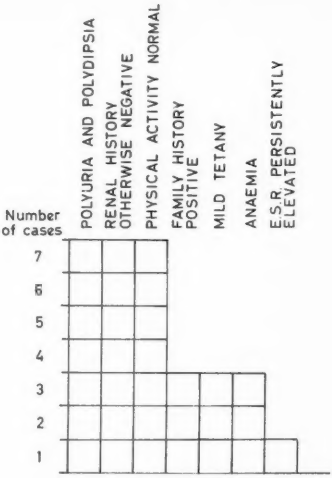

Fig. 1. Diagram of symptoms presented by seven patients with juvenile nephronophthisis on first admission to hospital.

reagent (12). Ammonia was estimated using the micro-diffusion technique with absorption in boric acid solution and titration with 0,1 *N* acid (4). Amino-acids in the urine were determined accordning to Dent (6). The biochemical methods have otherwise been such commonly used in routine clinical work.

History and Clinical Course

The reason for admission is listed in Table 1. It is noteworthy that in four or five cases there had been no complaint of disease.

The symptoms present before the first admission are given in Fig. 1. In each case

TABLE 2. *Sex distribution and family history in seven patients with "juvenile nephronophthisis".*

Case	Patients Sex	Born	Siblings Number	Sex	Born	State of health	Parents
1. R.A.	Female	3/2/1947	2	Female	1948	Good	Healthy
2. E.A.	Female	31/3/1946	1	Male	1941	Died with uraemia in 1947.	Father healthy. Mother op. because of renal tuberculosis.
			3	Female	1948	Healthy. Renal function normal.	
3. G.M.O.	Female	19/6/1946	2	Male	1948	Healthy	No renal disease
4. A.R.	Female	19/4/1944	2	Male	1948	Healthy	Healthy
5. K.P.	Male	11/8/1944	1	Female	1943	Pat. no. 6	Paternal uncle died 1923 at the age of seven in "diabetes".
			3	Male	1947	Healthy	
			4	Female	1951	Healthy	
			5	Male	1953	Healthy	
6. S.P.	Female	2/6/1943	See patient no. 5.				See patient no. 5.
7. V.S.	Female	18/1/1950	1	Male	1945	Healthy	No renal disease

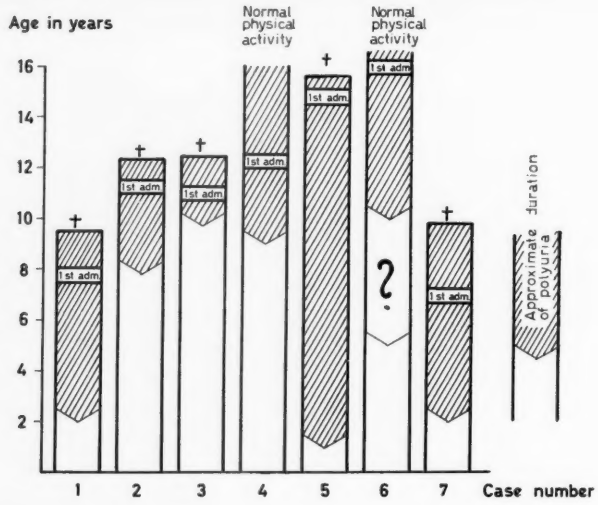

Fig. 2. Diagram showing duration of polyuria before first admission to hospital.

direct interrogation only revealed a history of polyuria and polydipsia present for several years (Fig. 2). This early and typical feature was easily over-looked since—as is almost invariably the case with this symptom—it had been neglected by both parents and patients. In none of the children affected was there any past symptom, except polyuria, referable to the urinary tract. The family history was positive in three patients (Table 2). Mild tetanic symptoms had occurred before the first admission in three patients and later appeared in one more. Moderate or severe anemia sooner or later developed in all patients.

The presence of polyuria but lack of alarming or disabling symptoms during the early years of disease thus was the most typical feature of the history.

Clinical Findings

Physical examination

The findings are graphically recorded in Fig. 3. The general condition on first admission was good in six of the patients.

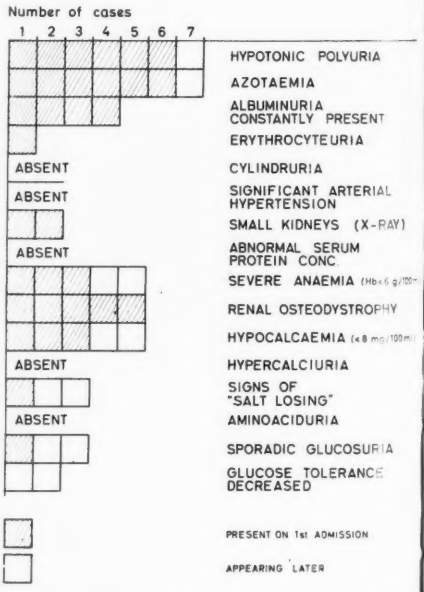

Fig. 3. Diagram showing the findings on clinical examination of seven patients with juvenile nephronophthisis.

TABLE 3. *"Spontaneous" variations of alkaline phosphatase activity.*

Case no.	Alkaline phosphatase activity (Buch units)	Time elapsed between determinations	Therapy between determinations
4	54		
	24	One week	None
	19.5	One week	None
	40	Two years	
6	45		
	23	Two weeks	None

Growth was somewhat retarded in four patients, normal in the others (Cases 2, 6, 7). There was no obvious correlation between the duration of polyuria and growth retardation. Blood pressure was normal or slightly elevated, the highest reading being 150/100. The eye-grounds were normal in six patients, but one showed Grade I–II hypertensive changes shortly before death. The children never developed oedema.

Plain roentgenograms of the abdomen showed both kidneys to be of small size in two patients (Case 3 and 5). The surface looked smooth and even. In the other patients the size of the kidneys seemed to be normal. Renal pelvices and ureters, in four patients visualized by intravenous urography or retrograde pyelography, showed no evidence of dilatation.

Urinary findings were sparse. Deposits were seen only in one patient who had a sporadic erythrocyturia not exceeding 2.5 million red cells per 12 hours. Albuminuria, when present, did not exceed 0.8 promille with the exception of one patient with a maximal urinary albumin concentration of 3 promille. Urine cultures were negative.

A moderate or severe normochromic or slightly hypochromic anaemia was observed in all patients. During periods of anaemia reticulocyte values between 2.3 and 5.0 per cent were observed in five severely azotaemic patients. Serum iron determinations disclosed normal levels in two out of three patients.

Skeletal disease was present in 5 patients, the serum alkaline phosphatase activity being elevated in all. In one marked rachitic changes were obvious on physical examination, in three there were on radiological examination osteoporotic changes beneath the epiphyseal lines, in one an increased serum alkaline phospha-

TABLE 4. *Characteristics of water metabolism.*

24-hour urine volumes	Usual range of specific gravity of 24-hour urine	Minimal specific gravity observed in random samples	Maximal specific gravity	Per cent excretion in 4 hours of a water load of 800–1000 cc (all patients azotaemic)
1500–3500	1.006–1.007	1.001–1.003 (5 pat.) 1.005–1.006 (2 pat.)	1.010–1.012[a, b] (5 pat.) 1.008–1.009 (2 pat.)	50–60 % (6 pat.) 100 % (1 pat.)

[a] Values above 1.010 were seen at a few exceptional instances in three patients. Administration of pitressin tannate i.m. did not improve the concentration capacity.

[b] A concentration capacity up to 1.015 was found in one patient (Case 7) before azotaemia was manifest.

TABLE 5. *Renal clearances and urine pH.*

Case	Renal clearance ml/min/ 1.73 sq.m		Renal clearance Lit./24 h/ 1.73 sq.m[a]		pH of random urine samples
1	C_{IN}	9			5.85
	C_{CR}	9			
	C_{PAH}	21			
2	C_{IN}	7			6.33
	C_{PAH}	8			
3	C_{CR}	11			5.5
4	1956 $C_{CR(exogenous)}$	35	1958 C_{CR}	48	See Fig. 5
5			C_{CR}	16	6.0
6			C_{CR}	40	5.5
7	1956 C_{Urea}	46 per cent	1958 C_{CR}	43	See Fig. 5

[a] For normal values in children see Winberg (20).

tase activity was the only evidence of osteodystrophy. Remarkably rapid changes in the phosphatase activity were noted in two patients (Table 3).

Renal Function Studies

Water metabolism

As already mentioned, polyuria was the leading symptom in all patients (Fig. 1–2). The main characteristics of water metabolism are summarized in Table 4.

Patient No. 7 who was first seen in the Hospital when her serum non-protein nitrogen level was still normal deserves a special comment. Her 24-hour urine volumes at that time amounted to between 700–1700 ml, with a specific gravity varying between 1.012–1.015. During the following three years the non-protein nitrogen level successively increased to about 150 mg per 100 ml, while the concentration of the 24-hour urine volumes gradually decreased to about 1.006.

Glomerular function

Azotaemia was—as a rule unexpectedly—found at the first admission in six

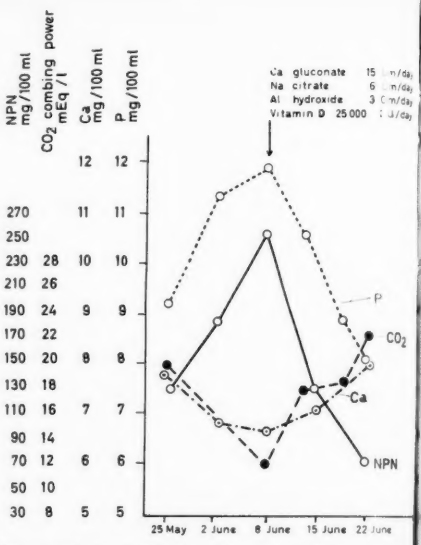

Fig. 4. Diagram showing the effect of treatment on serum carbon-dioxide combining power and the serum level of non-protein nitrogen, calcium and phosphate in Case 5.

patients. Despite the good general condition there was in several patients a remarkable reduction of the glomerular filtration rate (GFR) (Table 5). The clearance of PAH was extremely low showing but slightly higher values than that of inulin in two patients. Great variations of the non-protein nitrogen level were often seen (Fig. 4).

Renal tubular electrolyte regulation and acid-base stabilizing capacity

In three patients an abnormally high renal "salt loss' was suggested by low or falling plasma levels of sodium and chloride (Table 6).

A slight decrease in blood pH and CO_2-combining power was usually found. Occasionally a marked decrease of the latter to about 10 mEq per litre was observed

TABLE 6. *Sodium, chloride and potassium serum concentrations in three patients.*

Case	Sodium mEq/lit.	Chloride mEq/lit.	Potassium mEq/lit.
1	130	85	4.5
3	104	70	4.7
5	145	108	4.4
	132	96	7.0

(Fig. 4). When the patients were acidotic, this state seemed to be correlated to the azotaemia and the state of hydrationand not to be due to a specific distal or collecting tubular insufficiency with inability to conserve fixed cat-ions. This opinion is supported by the finding of a but slightly impaired capacity to acidify urine and to secrete ammonia in two patients given an acid load (Fig. 5). Even in the other five patients random urine samples showed pH values of 5.5–6.33 (Table 5), probably even in these cases excluding specific tubular insufficiency causing acidosis. Urinary calcium excretion is given below.

Amino-acid and glucose excretion

Amino-acid excretion, investigated in six patients, was found to be normal. A slight increase in proline excretion in one patient is hard to evaluate.

Sporadic glucosuria, never exceeding 1.9 per cent, was observed in three patients. Fasting blood sugar levels varied in two of these patients between 122–237 and 122–140 mg per cent respectively. Since in this last patient the glucose tolerance was abnormally low when tested by the double glucose tolerance test, the reason for the glycosuria most likely seems to be simple overflow.

TABLE 7. *Familial incidence of juvenile nephronophthisis.*

Authors	No. of families	Known consanguinity	Number of children Affected	Number of children Healthy
Faconi *et al.*	2	1	8[a]	2
Grüttner *et al.*	1	0	2	0
Hackzell *et al.*	2	0	4	4
Hooft *et al.*	1	1	2[a]	1
Present study	6	0	8	9
Total	12	2	24[b]	16

[a] The diagnosis was probable in one case, established in the others.

[b] Among those affected there were 13 boys and 11 girls. Thus the disease was equally distributed among both sexes.

Calcium and phosphate metabolism

The serum phosphate level was increased in all patients, the glomerular filtration rate being markedly reduced. At the same time there was a decrease of the serum calcium level in five of the patients. Repeatedly the 24-hour urinary calcium excretion amounted to 20–70 mg, only on two occasions showing higher, but still normal values, 150–240 mg.

Genetic considerations

Known genetic facts about the families of the present study and those reported earlier are shown in Table 7. In the affected families there were several more diseased children than healthy ones. In addition several of the healthy siblings had not reached the age at which the first symptoms appeared in the affected members of the family (Table 2).

The disease was equally distributed in both sexes. In hitherto published series there have been 11 girls and 13 boys (the two probable, although not established

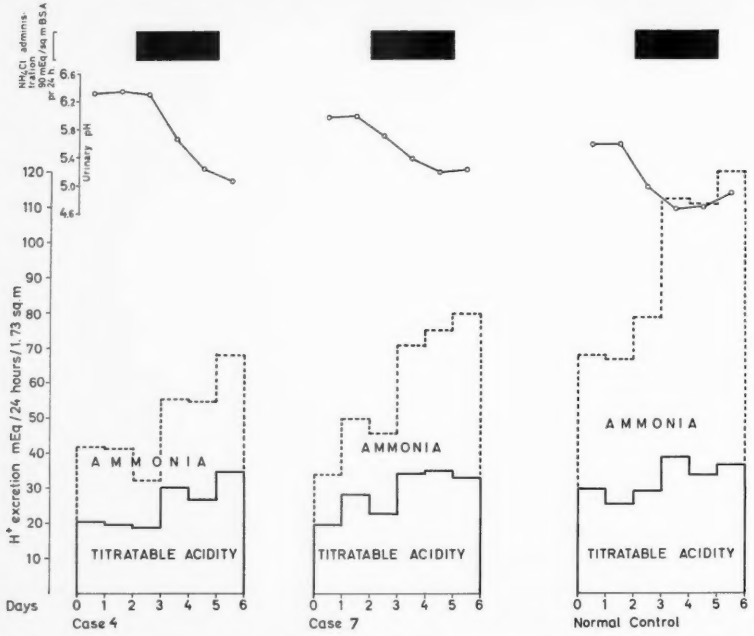

Fig. 5. Diagram showing the effect of an acid load on urine pH and the urinary excretion of titratable acidity and ammonium in Cases 4 and 7 and a normal control.

cases included, see Table 7). In five families both sexes were represented.

The data reported in Table 7 strongly favour the assumption that the disease is inherited as an autosomal recessive gene.

There is some evidence that the heterozygote state may be detectable in some instances since a reduced concentration capacity was observed in the father of one of our patients. He was apparently healthy at the age of 40 years. A similar finding was also reported by Grüttner *et al.* (10).

Discussion

In all patients the clinical symptomatology was very uniform and showed such close similarities to what has been described as typical of "familial juvenile nephronophthisis" (8, 10, 11, 14) that it seems possible to establish the diagnosis on clinical grounds even in the absence of a positive family history.

The early characteristic feature of the disease is polyuria due to a tendency to hypotonic urine secretion, a symptom which may be present for several years before any other symptoms of disease appear. In advanced cases the specific gravity of the 24-hour urine often is very low, but the urine volumes are never increased to the same extent as in true diabetes insipidus, probably due to the reduction of glomerular filtration rate.

Since the glomerular filtration rate may

be only slightly reduced even after the persistence of polyuria for several years (Case 7) renal tubular damage most likely precedes glomerular degeneration. The finding in two patients of inulin to PAH clearance quotients of about 1:1 and 1:2 respectively points to a marked reduction in the capacity of secreting PAH and is in accordance with the view of an ascending degeneration of the nephrons. That glomerular fibrosis represents changes secondary to tubular degeneration may explain why the urinary findings are so sparse despite an extensive reduction in glomerular filtration rate, i.e. the glomerular function ceases and hyalinization occurs when the nephron has become non-functioning. In this connection it may be pointed out that in chronic pyelonephritis where the primary damage is located in the distal parts of the nephron (18), and the glomerular lesions are probably secondary, albuminuria and erythrocyturia are also very discrete.

Defective water reabsorption is the only regular sign of renal tubular dysfunction. The low serum levels of sodium and chloride which were observed in some of the patients is most probably also the consequence of tubular damage. On the other hand, there is no convincing evidence of a selective distal tubular inability to "reabsorb bicarbonate" or to produce ammonia after an acid load. Pathologic aminoaciduria is not present, and it seems doubtful whether the glucosuria intermittently observed in three of our patients and in one of those described by Fanconi *et al.* (8) results from renal tubular damage alone, as pointed out earlier.

Osteodystrophy is one of the more prominent features of this disorder. The characteristics of the renal disease suggest that the skeletal abnormalities are pathogenetically connected with the azotaemic state and not primarily with renal tubular dysfunction as in the De Toni–Debré–Fanconi syndrome, cf. Dent & Harris (7). In two of our patients there were marked, rapid fluctuations of the alkaline phosphatase activity. The reason for these changes is not evident but may be due to rapid variations in skeletal mineralization as has been found in osteodystrophy complicating the azotaemic state (5).

The initial renal tubular dysfunction pattern present in the patients with this disorder may be termed "water-losing nephropathy" (17), a state which only during the last years has become more widely recognized as typical of several clinical entities. It is found in pyelonephritis (2, 16, 21), hyperparathyroidism (13), myelomatosis localized in the kidney (17), obstruction of the lower urinary tract not complicated by infection (17, 19), and familial nephrogenic diabetes insipidus (9). With the exception of the last, there is in these conditions a primary lesion of the distal tubules and/or the collecting ducts. Since the renal functional disturbance observed in early cases of juvenile nephronophthisis shows close similarities to that in the disorders listed above, it may be anticipated that also in juvenile nephronophthisis the primary lesion of the nephrons is localized in this particular area. It cannot, however, be excluded that the initial damage occurs in the loop of Henle since a normal urinary concentrating capacity presupposes that this part of the nephron is not damaged (1, 22).

Summary

Clinical data of seven patients with juvenile nephronophthisis are presented. The communication is an attempt to define the clinical characteristics of the disease and to discuss the etiology and pathogenesis of the symptoms.

The early characteristic feature of the disease is polyuria due to a tendency to hypotonic urine secretion. Because of a marked reduction of the glomerular filtration rate the urine volumes never become increased to the same extent as in true diabetes insipidus.

Defective water reabsorption is the only regular sign of tubular dysfunction. Low serum levels of sodium and chloride observed in some cases may also be the consequence of a tubular damage. There is, however, no convincing evidence of a selective tubular inability to "reabsorb bicarbonate" or to produce ammonia. Nor are there any signs of a proximal tubular damage.

Osteodystrophy is a prominent feature of the disaase. The skeletal abnormalities are pathogenetically probably connected with the azotaemic state.

The disease is genetically determined, and probably inherited as an autosomal, recessive gene. The heterozygote stage may be detectable.

An analysis of the symptoms and findings suggest that the primary lesion is localized in the distal tubules and/or collecting ducts or possibly in the loop of Henle. The course of the disease suggest an ascending degeneration of the nephrons.

Since the patients are in a good general condition and since albuminuria, erythrocyturia, oedema and arterial hypertension are absent or very discrete, a diagnosis of renal disease may be delayed for many years after the first appearance of symptoms.

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Juvenile Nephronophthisis. Part II

A Histologic and Microangiographic Study

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The renal changes in fatal cases of familial juvenile nephronophthisis have been described previously (Fanconi *et al.*, 1951, Hackzell & Lundmark, 1958, and Hooft *et al.*, 1959). The changes were regarded as being due to a diffuse renal contraction. No morphologic evidence of congenital defects in the kidney was given by any of these authors.

In the present paper, the renal pathology of two fatal cases of this disease will be described. The clinical features have been presented in the report by Broberger, Winberg & Zetterström in this issue (Cases 2 and 3). Evidence will be presented to show that there is a characteristic lesion of the descending and ascending limbs of the loop of Henle. This defect is associated with a cortical atrophy which is considered to be secondary to the tubular lesions. In view of the tendency of this disease to occur as a familial trait, it seems probable that the tubular defect has a congenital basis.

Material and Methods

The kidneys from two patients (both $12\frac{3}{12}$ years old) with the clinical picture of familial juvenile nephronophthisis with terminal azotemia were available for study (Cases 2 and 3 of Broberger *et al.*). Autopsy permission was restricted to an examination of the kidneys. A partial autopsy was performed 2 hours (Case 2) and 3 hours (Case 3) *post mortem*, respectively. One kidney from each case was kept intact in its capsule and immediately stored at −20°C. The other kidney was removed from its capsule, split open, and preserved in 10 % neutral formalin for histologic examination. The formalin-fixed left kidney from Case 2 weighed 40 g, and the fixed right kidney from Case 3 weighed 60 g (normal, 95 g). The gross appearance (Figs. 1 and 2) of both specimens was essentially similar. The fibrous capsule stripped off easily, the subcapsular surface was slightly granular and pink. In both specimens a few pinpoint-sized, thin-walled cysts were seen at the surface of the cortex. On sectioning, the cortex was found to measure 2–3 mm in thickness (normal 5 to 6 mm). The medulla measured 10–12 mm in thickness which is within the normal size range. Cysts were also found in the medulla, close to the corticomedullary junction as well as in the center of the pyramids (Figs. 1 and 2). Cortical cysts measuring 0.2–1.8 mm in diameter were present in both specimens (Fig. 2). The calyces, pelves, and ureters appeared normal. No malformations of the major renal vessels were present. The renal tissue taken from both specimens for histologic study was dehydrated, embedded in paraffin and stained with hematoxylin-eosin, van Gieson's connective tissue stain, Verhoeff's elastic tissue stain (counterstained with van Gieson's

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stain), Lendrum's stain for reticulin, and alcian blue-PAS according to McManus & Guerrero.

The two frozen kidneys were used for microangiography (Bellman) employing a technique modified for the infantile kidney (Ljungqvist). This procedure was carried out after 18–21 months of storage, which unfortunately resulted in impairment of the histologic detail. The ureter was injected with a 25 % solution of Urografin®, using slight pressure, and roentgenograms were taken. Following this the contrast medium was removed, the pelvis washed with saline, and the renal artery injected with a 10 % aqueous mixture of Micropaque® for four hours. An initial pressure of 110 mm Hg was increased to a final injection pressure of 175 mm Hg. After stereo-X-ray photographs had been taken, the gross specimens were sliced, embedded in paraffin, and prepared for stereo-microangiography. After this procedure samples were sectioned serially at 5 μ. The sections were stained with Verhoeff's elastic tissue stain counterstained with van Gieson's stain. A few sections were stained with alcian blue.

Histologic Findings

The histologic findings differed in degree but were essentially similar in the two cases. In the kidneys from Case 2 the cortex was thinner and appeared to contain a larger number of hyalinized glomeruli, while the cortex from Case 3 displayed more numerous cortical cysts.

Renal corpuscles. Two basically different types of degeneration were noted, (a) hyalinization, and (b) cystic dilatation. Varying degrees of hyaline change were present in most of the glomeruli, although a small number were apparently normal. As was noted by previous observers, in those renal corpuscles which showed minimal change, a thickened basement membrane was seen only in the parietal layer, while the glomerular tuft appeared to be unaffected. In some instances the thickend parietal layer of Bowman's capsule stained a deep magenta with the alcian blue-PAS, denoting the presence of neutral mucopolysaccharides. In more severely affected corpuscles, the thickened basement membrane involved the glomerular tuft as well, and many extreme cases were found in which the lumen was completely obliterated. It was interesting to note that only rarely did the completely scarred glomeruli stain magenta with the alcian blue-PAS.

The cystic glomerular change was present in the subcapsular layer. In serial sections all the cortical cysts were found to contain glomerular tufts of varying degrees of atrophy. For this reason they were considered to be dilated renal corpuscles. The parietal layer of these cysts varied in thickness, some cysts having thick fibro-elastic walls, others displaying thin reticulin sheaths. None of them, however, stained positively with the alcian blue-PAS. No correlation could be found between the structure of the parietal wall of these cysts and either their size or the degree of atrophy of the glomerular tuft. These cysts were regularly spherical and did not communicate with any part of the uriniferous tubules, although atrophic nephric tubules could be identified in their immediate vicinity (Fig. 3).

Proximal convoluted tubules. In addition to the previously mentioned atrophic proximal convuluted tubules, hypertrophic tubules were found arising from the glomeruli which showed minimal changes. Occasional groups of proximal tubular cells displayed hyaline droplet change. The granules were PAS-positive. The al-

cian blue-PAS technique also brought out occasional PAS-positive thickening of the basement membrane, especially in the neck portion of the tubule.

Descending limbs of the loop of Henle. This portion of the nephron exhibited the most characteristic and marked change in these specimens, in that they were moderately enlarged and strikingly convoluted (Fig. 4). There was considerable variability between different nephrons with respect to the degree of this convolution (see below). The size and configuration of the individual cells appeared to be within the normal range. The basement membrane was slightly thickened, and PAS-positive. The *thin segments* had a moderately thickened basement membrane. Except for this and an occasionally tortuous course, they appeared essentially normal.

In occasional instances the flexure of Henle's loop was dilated. In extreme cases this had the appearance of a small ovoid medullary cyst of approximately 2 mm in diameter. A study of serial sections confirmed the fact that one descending and one ascending limb of Henle's loop opened into the cortical aspect of each cyst. As is seen in Fig. 5, the descending limb with its thickened basement membrane may project nozzle-like into the cyst. The ascending limb of Henle's loop sometimes leaves the cyst in a tangential manner. This configuration might have acted as a valve, and prevented free passage of fluid from the cyst further along the nephron.

Ascending limbs of the loop of Henle. The ascending limbs—whether or not they were connected with a medullary cyst—showed a varying degree of coiling with thickening of the basement membrane. This membrane was massive, homogeneous and PAS-positive (Fig. 6). In some cases the segment consisted of a thin cellular cord surrounded by a PAS-positive sheath, while in other instances no remnants of the parenchymal cells could be seen, and the segment was represented by a coiled strand of PAS-positive material. It seems reasonable to assume that these findings represented stages in the progressive obliteration of this segment of the nephron.

Distal convoluted tubules. This portion of the nephron displayed the same range of morphologic characteristics as were seen in the ascending limb of Henle's loop.

Collecting ducts. From the arched collecting tubules to the papillary ducts, the collecting duct system showed no apparent abnormalities. The papillary ducts, however, frequently exhibited a variable degree of dilatation. The lumen of such an ectatic portion was in continuity with a minor calyx. These dilatations were fusiform and characteristically lined with a tall, columnar, stratified epithelium. Other similarly lined spaces in the papillary zone of the medulla were essentially spherical and measured up to 8 mm in diameter. From a study of serial sections these were found to have no connection with either straight collecting tubules or with minor calyces. In view of the essential identity in histologic structure it seems reasonable to assume that these cysts were derived from papillary ducts. In contrast to the medullary sponge kidney (Ekström *et al.*) these cysts did not contain calculi.

Pelvis and calyces. The pyelograms of the specimens showed no abnormality in gross morphology of pelvis or calyces. There was no filling of the medullary cysts or ectasies. The histologic structure of the

wall of the pelvis and calyces was normal.

Stroma. The increased interstitial fibrous tissue contained foci of plasma cells and lymphocytes as previously described for this condition. The arteries had moderately thickened media and occasionally there was a slight intimal thickening. The elastic laminae appeared normal. The adventitia of a few archiform arteries contained thick sheaths of circularly arranged smooth muscle. This was occasionally in continuity with the calyceal musculature. Very occasional interlobular arteries exhibited a similar but thinner muscular adventitia. Within this adventitial smooth muscle were strikingly developed venous sinusoids.

Microangiographic Findings

Cortex. The vascular pattern is seen in Figs. 7–8. The thinning of the cortex is represented by a reduced vasculature, a tortuosity of the interlobular arteries and afferent glomerular arterioles (Fig. 8). The tortuosity was most apparent in the long juxtamedullary afferent glomerular vessels. The cystically enlarged renal corpuscles were never filled. Nor could these cysts be localized on the basis of the vascular pattern. As a rule, the peritubular capillaries were not filled.

Medulla. The efferent arterioles from the juxtamedullary glomeruli and the vasa rectae could easily be visualized. Usually, the vasa rectae were straight, although occasionally they were sinuous, only rarely were they markedly tortuous. No aneurysms were demonstrated, nor was there any direct filling of the medullary cysts.

Occasionally, glomerular capillaries ruptured during injection. This allowed the radioopaque material to fill the nephron, although only rarely did this injection extend beyond the loop of Henle. Thus it was possible to study from stereo-microangiograms the relationship of the vasculature to the nephron especially in the medullary region. The unexpected observation was made that an *uncoiled* blood vessel was intimately associated with a markedly *coiled* descending limb of Henle's loop, so that in the microangiographic projection they have the appearance of the Esculapian serpent (Fig. 10). Occasionally this filling of the nephron resulted in the filling of a small medullary cyst confirming the fact that such cysts are dilatations of the flexure of Henle's loop (Fig. 9).

Measurements of the Descending Limb of the Loop of Henle

In view of the striking convolution of the descending limb of Henle's loop, and the fact that the medulla was normal in thickness, some approximate measurements seemed warranted. Tracings of such convoluted segments as visualized in the microangiograms were made by means of a camera lucida at a magnification of × 67. From such tracings the overall length of the descending limb was measured. This length was compared to the length of the straight line between the end points of the descending limb. This ratio in a markedly convoluted segment was 3.3. Thus the coiling involves an actual increase in length and is not a result of mechanical distortion. The diameter of the lumen and of the cross-sectional diameter inside the basement membrane were measured for a series of abnormal descend-

ing limbs. Similar measurements were made from a normal kidney from a 12-year-old boy. Since in both instances the segment is roughly circular in cross section, the area of the parenchyma could be calculated. A comparison of such cross-sectional areas indicated that the abnormal segment had a parenchymatous cross-sectional area 3.5 times greater than the normal. Thus, it appears that the volume of parenchyma of a convoluted descending limb of Henle may be as much as 11 times greater than normal. Since the size of the parenchymatous cells were of the same order in the normal and abnormal specimens, it would appear that in this condition there is a hyperplasia of the descending limb of the loop of Henle.

Discussion

The morphologic findings in kidneys from patients displaying the clinical features of familial juvenile nephronophthisis as have been reported previously may be summarized as follows. A diffuse contraction of the kidney causing coiling of the nephrons, an ensheathing of the nephrons by a characteristically thickened basement membrane, an initial thickening of the basement membrane of the parietal layer of the renal corpuscles eventually progressing to complete hyaline degeneration of the entire corpuscle.

In both cases described in this report the kidneys were reduced in size and weight. However, this reduction of the renal parenchyma appeared to be restricted to the cortex, which was markedly thinned. The thickness of the medulla, on the other hand, was within the normal range.

Within the cortex both the interlobular arteries and the afferent glomerular arterioles were markedly distorted and convoluted. This would seem to have been

Plate I.

Fig. 1 and 2.—Kidney of Case 2, sliced at different levels after formalin fixation. Note small medullary cysts (from flexures of Henle's loops), large medullary cysts (from papillary ducts) and, in Fig. 2, a single cortical cyst. Note also the reduced thickness of the cortex.

Fig. 3. Cortical (gomerular) cyst from Case 2. Note atrophic glomerular tuft and protein-like material in capsular space. Atrophic tubules showing a positive PAS reaction can be seen in the lower part of the figure (Alcian blue–PAS: × 100).

Fig. 4. Markedly coiled descending and ascending limbs of Henle's loops. The latter show thickened basement membranes (Alcian blue–PAS: × 100).

Fig. 5. A small medullary cyst formed from the flexure of Henle's loop. Note the tortuous limb approaching the cyst from above, and the nozzle-like opening of the descending limb into the cyst. The ascending limb leaves the cyst tangentially from its upper left aspect. The epithelial lining of the cyst is artificially separated from the basement membrane (Verhoeff–van Gieson: × 100).

Fig. 6. A moderately tortuous ascending limb of a loop of Henle, showing a thick PAS–positive basement membrane (Alcian blue–PAS: × 240).

Plate II.

Fig. 7. Microangiogram of a frontal section from kidney, Case 2 (700 μ). The thinned cortex is poorly vascularized. The medulla shows vasa rectae and occasional nephrones. The large medullary cysts are not filled. Some small medullary cysts are filled, but cannot with certainty be distinguished at this magnification (× 1.5).

Fig. 8. Microangiogram of cortex showing distorted and tortuous interlobular arteries and afferent glomerular arterioles. Note the more apparent tortuosity towards the peripheral parts of the interlobular arteries (upper aspect) and of the long afferent arterioles of glomeruli in juxtamedullary position (central and lower aspects) (× 25).

Fig. 9. Descending and ascending limbs (arrows), and cystic enlargement of the flexure of Henle's loop, forming a small medullary cyst. Minimally coiled vasa rectae to the left of the limbs (× 25).

Fig. 10. Microangiogram of medulla showing markedly coiled descending limbs of Henle's loops. Note the intimate association with essentially straight vasa rectae (× 25).

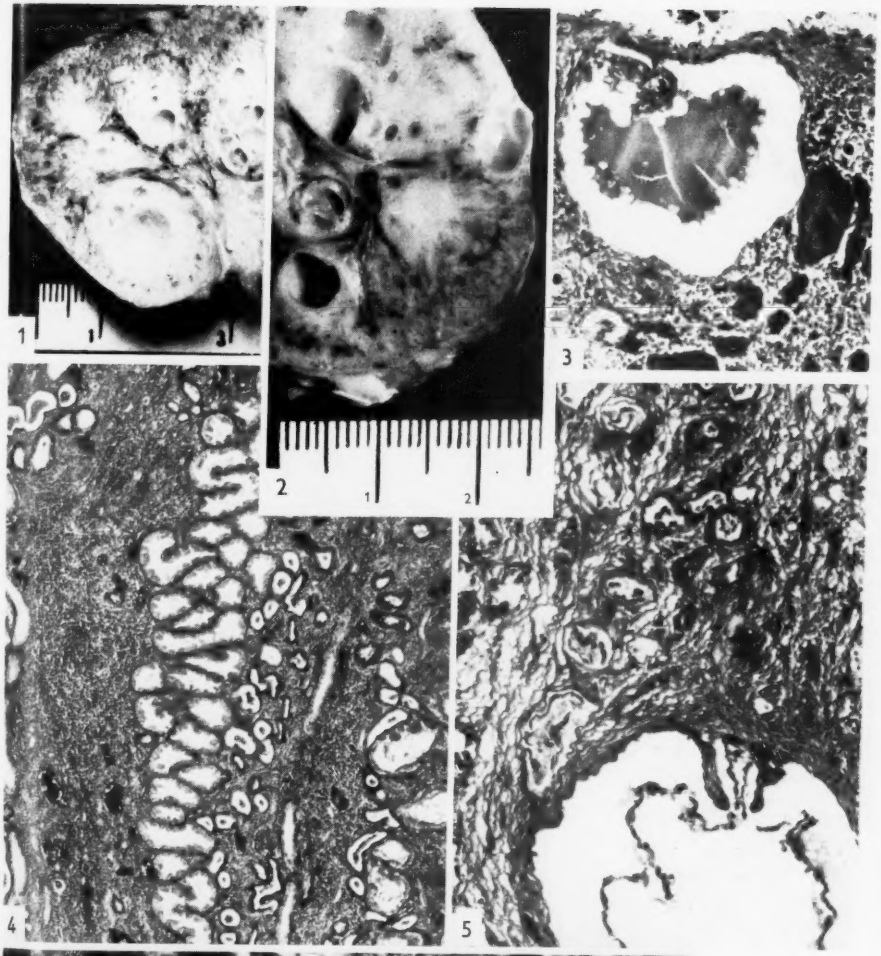
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Plate II.

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the inevitable result of a progressive thinning of the cortex. Many stages of hyaline degeneration of renal corpuscles were found. In this respect these two cases conform to the characteristics described in previous reports, but they differ, however, in that some renal corpuscles showed cystic enlargement with atrophy of the glomerular tuft. None of these glomerular tufts or cysts whether studied in microangiograms or in histologic sections, could be demonstrated to contain contrast medium. It seems reasonable to assume, therefore, that at the time of death they were essentially avascular.

Although there was a characteristic thickening of the basement membrane of all segments of the nephrons, it was relatively more massive in association with the ascending limbs of Henle's loops and the distal convoluted tubules. In extreme cases the lumen of the ascending limb of Henle's loop was obliterated. This more or less complete blockage may have resulted in sufficient back-pressure to have initiated the cystic dilatation of the flexure of the loop which was found in these two specimens. The characteristic tangential relationship of an ascending limb of Henle's loop to such a cyst would act as a valve and further interfere with free passage of fluid along the nephron, causing further enlargement of the cyst. This cystic feature has not been described previously in cases of familial juvenile nephronophthisis. The occurrence of these renal cysts in the two patients of the present study may be explained by the fact that they were older than those of previous studies.

The striking tortuosity of the limbs of Henle's loops cannot have been due to a diffuse contraction of the renal parenchyma in these two cases. In the first place, the medulla did not exhibit any shrinkage. Moreover, it seems that any generalized change in the dimensions of the medulla would affect equally all structures lying in it.

If the coiling of the descending limbs of Henle's loops is not the result of compression of the medulla, then it must be due to an actual increase in their length. Quantitative estimations made on such nephric segments showed that this was actually the case. The length of a markedly convoluted descending limb of the loop of Henle was three times greater than normal, while the volume of its parenchyma exceeded that of a normal control by tenfold. The convolutions shown by the vessels in the cortex seem to be secondary to the thinning of the cortical layer, and not to reflect a hyperplasia.

The histologic structure of the walls of the large cysts in the renal papillae was indistinguishable from that of the ectatic papillary ducts. In fact these two structures could be distinguished with certainty only from a study of serial sections. It seems probable that the cysts represent ectatic areas of papillary ducts which secondarily lost their connection with the collecting duct system. It would be unprofitable to speculate, from the material available, concerning the mechanisms involved in this sequence of events.

The high incidence of this rare disease in certain families has led previous investigators to regard it as being genetically determined. The two cases reported here throw no light on this aspect of the problem. Their clinical history is compatible with the assumption that the primary lesion was a slowly progressing dis-

turbance of the function of the more distal part of the nephron. This is supported by the fact that the distal half of the nephron was more severely affected than was the proximal half. It seems probable that the medullary cysts found in these cases as well as the changes in the renal corpuscles were secondary to the thickened basement membranes. It is not possible to determine whether the latter is a primary stromal defect, or is secondary to a biochemical abnormality of the parenchyma of the nephrons themselves. It does seem clear, however, that the distal half of the nephron was initially hypertrophic prior to the massive increase in its basement membrane, since even the completely atrophic ascending limbs of the loops of Henle showed a convoluted course analogous to that followed by the convoluted descending limbs of Henle's loops.

Summary

The kidneys from two cases of familial juvenile nephronophthisis were studied grossly, in histologic sections and by means of stereo-microangiography. The clinical history of these same cases is reported in the preceding paper in this issue (Broberger, Winberg & Zetterström).

The kidneys from these two individuals showed essentially the same characteristics:

1. Their weight was significantly less than normal.

2. Their cortex was thinned with distortion and convolution of the interlobular arteries as well as of the afferent glomerular arterioles.

3. Most of the renal copuscles showed varying degrees of hyaline degeneration, although some were cystically enlarged and their glomeruli were atrophic.

4. The nephric tubules were ensheathed in a thickened, PAS-positive basement membrane. This condition was more marked in the distal half of the nephron.

5. The medulla showed no decrease in thickness and contained characteristically convoluted limbs of Henle's loops. The descending limbs were shown to be hypertrophic. The convoluted character of the ascending limbs suggests that they too may have undergone hyperplasia prior to the massive thickening of their basement membranes.

6. The flexures of Henle's loops were occasionally cystically dilatated.

7. The papillary ducts exhibited occasional ectatic portions, which in some instances had progressed to form cysts.

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Blood Flow in the Foot and Calf of the Newborn

A Plethysmographic Study

by OLOV CELANDER

During recent years the changes in circulation at birth have been subject to numerous studies. For reviews see Young (10), Dawes (6) and Lind (8). The majority of these studies have been concerned with pressure, flow and oxygen determination from the heart and the central vessels, whereas the peripheral circulation seems to have attracted less interest. The change from foetal to extrauterine life involves a more drastic shift in circulation than most individuals are likely to encounter during the rest of their lives. Shunt mechanisms, which during foetal life make the two ventricles work in parallel, now close, separating the pulmonary circulation with its low pressure from the much higher pressure existing in the systemic circulation. The placental blood flow, which according to Dawes (6) accounts for more than half the combined cardiac output, suddenly disappears. Due to variations in the placental transfusion the total amount of circulating blood may vary considerably from child to child. To all these changes can be added the asphyxia which the child must withstand, even at an uncomplicated delivery, and the new demands for circulatory control mechanisms in response to environmental temperature changes and to hydrostatic influences. If circulatory stability is to be maintained by the newborn these changes will probably, to a great extent, engage the various physiological control mechanisms which normally regulate the cardiovascular system.

In the neonatal period measurements of the arterial systolic pressure have been applied to study how these circulatory adjustments are reflected upon the systemic circulation (1, 2). However, it cannot be taken for granted that a "normal" blood pressure guarantees a normal circulatory state. In the Poiseullie formula (pressure = C × flow × resistance), which can be applied to the systemic circulation as a whole or to any part of it, the variables which show quantitatively important changes during normal and abnormal circulatory adjustments, are flow and resistance.

This is the first in a series of studies in which the blood flow in the foot and calf has been measured during standard conditions in order to gain information about the circulatory state of the newborn, and the general trends in the physiological control mechanisms to which peripheral blood vessels are subordinated.

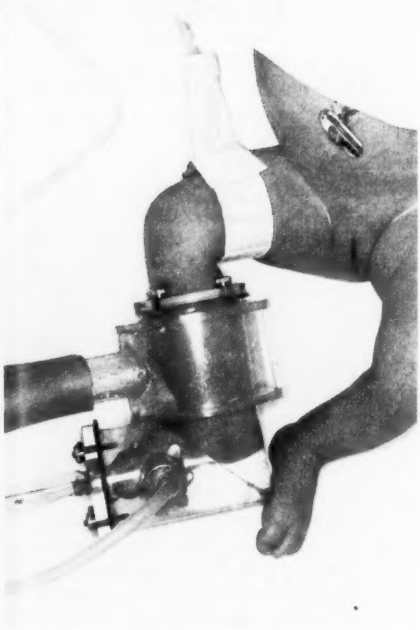

Fig. 1. Water-filled venous occlusion plethysmograph for the newborn ready for use.

Methods

The principles of venous occlusion plethysmography have been discussed by Barcroft & Swan (3). In short, the procedure is to enclose the extremity in a plethysmograph connected to a volume recorder. The fitting of the plethysmograph must be close enough to prevent leakage, but, at the same time, not so tight that the venous return from the extremity is impaired. The checking of this vital point will be discussed later. A sphygmomanometer cuff is placed proximally to the plethysmograph and during the actual measurements the cuff is inflated at a pressure below the diastolic arterial blood pressure. The venous return from the extremity will then be temporarily arrested, whereas the arterial inflow will be uninfluenced. The net result is a progressive increase in volume and the rate of arterial inflow can thus be determined. During recent years venous occlusion plethysmography has been successfully applied in a vast number of circulatory studies in adults.

The design of the plethysmograph used in this study is shown in Fig. 1. It can be described as a boot made of Perspex. To control leakage and also in order to keep the enclosed extremity at constant temperature conditions, the plethysmograph has been filled with water. Around the upper part the plethysmograph was supplied with a mantle into which hot or cool air could be directed by attaching polythene tubes. The air was supplied by an electric hair-drier thermostatically controlled by a thermometer in the distal front plate of the plethysmograph. A chimney connected the plethysmograph to the volume recorder, and the level of water was kept just at the base of this chimney. The volume recorder was a 5 ml piston recorder (Palmer), which had to be trimmed to perfection. The excursions of the volume recorder were calibrated from a tuberculine syringe, and the volume-recording system was sensitive to changes from 0.02 ml.

The plethysmograph must be fitted to the extremity with great care. Fig. 2 shows how a perfect sealing can be achieved. After measuring the circumference of the calf a Perspex ring (*A*) was chosen from a great variety of such rings to fit the calf snugly. In order to give as large a supporting area as possible the ring was made 5 mm thick. *B* in Fig. 2 is a 1-mm-thick rubber membrane with a circular hole fitting the circumference of the calf closely. This membrane is continued

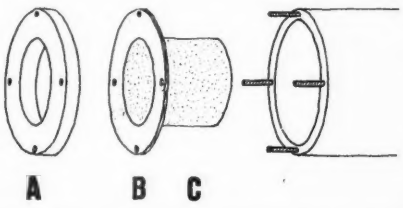

Fig. 2. Schematic drawing showing details of the sealing of the plethysmograph to the calf of the newborn. *A*, 5-mm-thick Perspex ring. *B*, 1-mm-thick rubber membrane with attaching collar (*C*) made from latex rubber.

into a very thin rubber collar (*C*), which like a cut stocking enclosed the calf of the child. This collar was made from rubber condoms. As mentioned, leakage could easily be controlled after filling the plethysmograph with water.

The sphygmomanometer cuff, made from rubber drainage tubing and adhesive plaster supported by ordinary paper clips, could be momentarily filled to any predetermined pressure from a pressure reservoir connected to a mercury manometer. Whether any obstruction to the blood return occurred at the point where the plethysmograph was sealed to the calf could be decided by determining the range of collecting pressure in the cuff over which blood flow records were obtained. If, for example, no regular increase in leg volume could be recorded at collecting pressures below 30 mm Hg, this was obviously due to the fact that an equal obstruction to the venous return was exerted at the point where the plethysmograph was sealed to the calf. In this series a collecting pressure of 40 mm Hg was used during the actual measurements and was found to give optimal blood flow records. Once asleep, most children tolerated the rapid filling of the sphygmomanometer cuff without being awakened or showing any startled response. In several children an arterial occlusion could be maintained for periods up to four minutes in order to induce reactive hyperaemia. Other children were more easily disturbed and the inflation of the cuff had then to be more gradual. Each collecting period lasted some 5 sec. They were repeated at intervals of 20 to 30 sec, and in most children a great number of separate observations of blood flow could be made.

The experimental conditions were standardized as far as possible. Body temperature of the children was measured and before the experiment the subject was taken into the laboratory, which was thermostatically maintained at 23°C, for about a quarter of an hour. All observations were made while the infant was lying in an incubator at 30°C. The relative humidity was 60 to 80 %. The lower part of the child was uncovered. After fitting the plethysmograph to the extremity the plethysmograph was filled with water at a temperature of 35°C. The volume of water was measured. The temperature in the plethysmograph was then kept at 32°C by the means already described. All observations were made while the infants were in natural sleep. If possible, the experiment took place after a feed and in a few cases a small extra feed was given from a bottle as the only sedative. Crying and struggling did occur, but sooner or later the infant accepted the situation and fell asleep. When the observations had been completed, the plethysmograph was emptied and the volume of water was again measured in order to determine the volume of the tissues enclosed in the plethysmograph.

Material. The subjects were 15 healthy, full-term infants of 2990 to 3750 g weight. In 14 cases the delivery was vaginal, one child was delivered by caesarean section. All deliveries had been considered free of complications. On clinical examination nothing abnormal was noticed and special care was taken to exclude abnormalities in respiratory and circulatory functions. Each subject was examined on two occasions, the first during the first day of life and the second at an age of 4 to 7 days.

Results

Fig 3 shows the blood flow tracings from a full-term, normal infant examined the first day of life. Arterial pulsations were reflected in the plethysmograms, showing that the volume-recording system was sensitive to the minute changes in leg volume which accompanied the arterial pulse wave. The arrows mark the instants at which the sphygmomanometer cuff was inflated at a pressure of 40 mm Hg. When the collecting pressure went on the record showed a small jump. This is due to the fact that the rapid filling of the cuff displaced a small amount of tissue

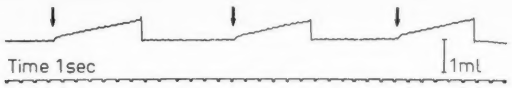

Fig. 3. A representative tracing of the blood flow in the foot and calf of a full-term normal infant six hours of age. Note the displacement artefact and the presence of arterial pulsations in the record. Blood flow 6.2 ml/100 ml/min.

into the plethysmograph. In other experiments this displacement artefact was more prominent. The persistence of pulsations in the tracings illustrates the well-known fact that the arterial inflow normally occurs in a pulsatile fashion. After some 6 sec the kymograph was arrested, whereafter the cuff pressure was released. The obstructed venous return from the extremity then freely drained, and the tissues in the plethysmograph returned to basal volume. After an interval of some 30 sec the observation was repeated. During the collecting periods the leg volume increased in a linear fashion. The rate of arterial inflow remained unchanged at 6.2 ml/100 ml/min. The relative increase of leg volume during the short collecting periods was less than 1 %. In some easily disturbed subjects the cuff could not be filled momentarily and this fact, together with the presence of a displacement artefact, sometimes distorted the linear slope in the plethysmograms during the earlier fraction of the collecting periods. In the calculations, therefore, the slope was determined from the end of first to the end of the fourth sec as a standard procedure.

In Fig. 4 the blood flows in this series at the two separate occasions are plotted against age. Each dot in the diagram represents the individual mean flow from, on the average, five consecutive observations. As shown in Fig. 3 the replicability of the observations was great, and, although slight spontaneous variations did occur in other subjects, they did not exceed 10 % of mean flow, provided that the experimental conditions were constant and that

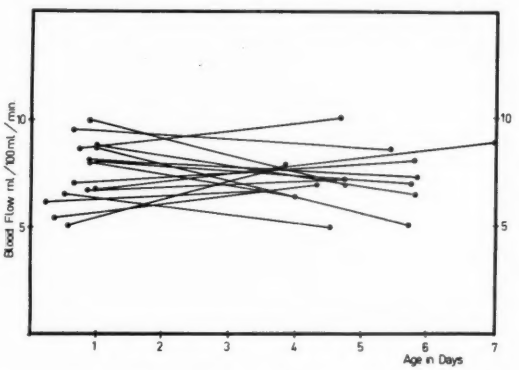

Fig. 4. Individual means of blood flow in the foot and calf of 15 full-term normal infants plotted against age. Mean blood flow in the group as a whole at the first occasion 7.9 (±1.7) ml/100 ml/min and at the second occasion 7.3 (±1.4) ml/100 ml/min.

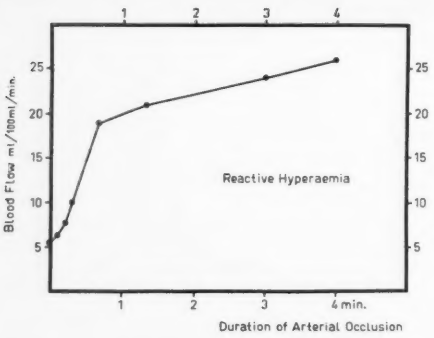

Fig. 5. Reactive hyperaemia in a full-term infant three days of age. The periods of circulatory arrest were successively prolonged from 5 sec to 4 min. Note the large circulatory reserve in the foot and calf in this subject.

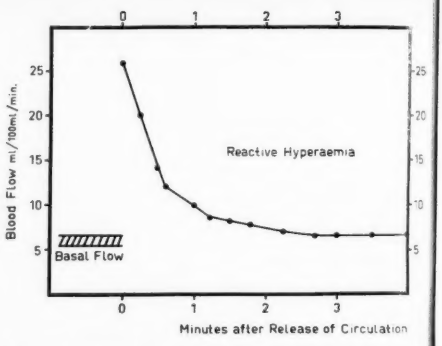

Fig. 6. Blood flow in the foot and calf the first four minutes following the release of circulation after a 4-min period of arterial occlusion. Note the rapid return to basal flow.

the child remained asleep. In this series of 15 normal, full-term infants the mean flow of the foot and calf was 7.9 ($\pm$ 1.7) ml/100 ml/min on the first examination, which, in most cases, took place during the second half of their first day of life. Blood flow was probably less in those infants which were examined during the earlier part of the first day. The adjustments which occur during the first hours of life will be the subject of a separate study. At the second occasion, later in the neonatal period, mean blood flow was 7.3 ($\pm$ 1.4) ml/100 ml/min. There was thus no statistically significant difference between the mean blood flows at the two separate occasions.

The labile character of peripheral blood flow was studied by inducing reactive hyperaemia. At these observations the cuff was inflated almost instantaneously from the pressure reservoir to a predetermined pressure of 150 mm Hg and a circulatory arrest was maintained for different periods up to four minutes. The arterial inflow was then recorded after a rapid deflation of the cuff from 150 to 40 mm Hg. Fig. 5 shows the vascular responses in a consecutive series of circulatory arrests from 5 sec to 4 min. Already after 20 sec of arterial occlusion, peak blood flow immediately after the release of circulation was about twice basal blood flow. After a 4-minute period of circulatory arrest, peak blood flow was approximately 27 ml/100 ml/min. This fivefold increase of blood flow reveals the huge potential local circulatory reserve in this area. Fig. 6 refers to the same infant and shows that, once the circulation was released, blood flow rapidly returned to basal level. There are several interesting features in reactive hyperaemia in the newborn, which will be reported on in a later communication.

Whereas normal full-term infants attain stability in the blood flow of the foot and calf quite soon after birth, this is frequently not the case in children which have been subject to pathological delivery. Only one child from this group will be commented upon here (Fig. 7). Because of a pro-

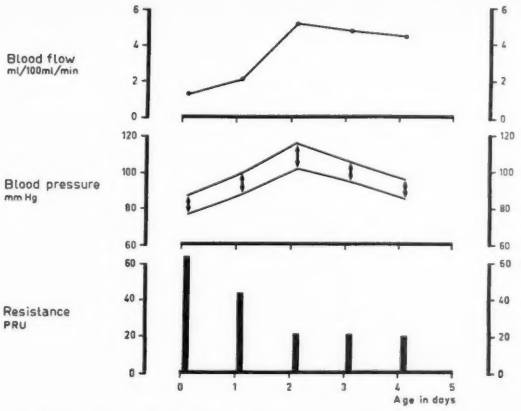

Fig. 7. Changes during the first days of life in systemic blood pressure, blood flow and peripheral resistance to flow in the foot and calf of a postmature infant delivered by the vacuum extraction method. See text.

longed gestation period (44 weeks) and insufficient labour this delivery was finished by use of the vacuum extraction method, which has lately been commonly used in Sweden. Although there were no gross signs of cerebral damage the baby was probably slightly irritable for the first two days of life. This child was subject to plethysmography at five separate occasions in the neonatal period. Concomitantly to the blood flow observations, the systolic and diastolic arterial blood pressure was determined by the plethysmographic method, which has recently been suggested by Celander & Thunell (5). On the first occasion, which took place three hours after the delivery, the blood flow in the foot and calf was low (1.3 ml/100 ml/min). It was still low 24 hours later (2.1 ml/100 ml/min) and not until two days after birth did the blood flow reach a more normal value (5.2 ml/100 ml/min). Thereafter it remained about this level. From the flow and pressure determinations the peripheral resistance to circulation in the foot and calf can be computed and expressed in "Peripheral Resistance Units" (7). As seen in Fig. 7, peripheral resistance was initially high (64 PRU) but gradually decreased and on the three last examinations peripheral resistance was only approximately one third of the initial (22, 22 and 21 PRU). There are good reasons for assuming that behind this initial high resistance to flow lies an active vasoconstriction effected by the sympathetic nervous system.

Discussion

Previous studies of the peripheral circulation in the newborn have been mainly indirect. During recent years Brück and co-workers (4) have successfully applied the heated thermocouple in the study of skin blood flow and the regulation of body temperature. Riley (9) used venous occlusion air plethysmography to study the blood flow in the upper extremity in full-term and premature infants. However, he

met with several experimental difficulties, mainly in controlling leakage of air from the plethysmograph. His statement, that the range of collecting pressure over which blood flow records were obtained lay between 60 and 80 mm Hg, must imply that a great deal of obstruction to the venous return occurred at the point where his plethysmograph was sealed to the baby's arm. In addition, such high occluding pressures might have interfered with the arterial inflow if the cuff pressure exceeded the diastolic arterial pressure. The facts that his observations were made while the subjects were lying in their ordinary cots in a room without temperature or humidity controls, and that it was not always possible to get the infants into a basal resting state, probably explain the low replicability of observations in many of his subjects. In spite of this criticism Riley was probably the first investigator of the peripheral circulation in the newborn who made a serious attempt to measure quantitatively the rate of peripheral blood flow.

In the present study the experimental difficulties were few, once technical details in sealing the plethysmograph and trimming the volume-recording system had been mastered. This can mainly be attributed to the choice of the lower extremity instead of the upper one. For anatomical reasons the plethysmograph could not easily be rejected by the child even by rather violent struggling.

The range over which the individual flow values varied in 15 mature infants lay between 5 and 10 ml/100 ml/min with a mean of 7.9 ($\pm$1.7) during the first day of life and 7.3 ($\pm$1.4) later in the neonatal period. The rates of blood flow in the hand and forearm for full-term infants, reported by Riley, fell within the limits of 1–7 ml/100 ml/min with a mean of 5.2 (S.D. 1.2). A comparison between the two groups is difficult since the experimental conditions were not standardized in the latter series. Neither would a comparison with the normal adult range be justified since the relations between skin and skeletal muscle differ widely in adult and newborn. The surface area of a smaller subject is relatively greater than that of a bigger subject of the same shape. This means that the proportion of skin in the foot and calf of the newborn is greater than in the adult. Furthermore, skeletal muscle in the adult makes up some 45 % of total body weight, whereas the corresponding figure in the newborn is 18 % (personal communication from Dr. M. Widdowson). The newborn, therefore, will have a larger proportion of skin in relation to skeletal muscle. The circumstance that the blood flow in the foot and calf during resting conditions might be less in mature infants than the normal adult range should not be taken as evidence that the peripheral circulation in infants is poorer due to incomplete development. Such a comparison can only be made after the potential circulatory reserves have been explored by inducing reactive hyperaemia, and even then the comparison would be invalidated by the predominance in the infant of skin in relation to skeletal muscle.

The present study was undertaken to determine the range over which the blood flow in the foot and calf varies in normal mature infants during standard conditions, and to find out how soon after birth the peripheral circulation in an extremity reaches a stabilized state. In

answer to the latter question, stability appears to be attained early in normal subjects, as the blood flow during the second half of the first day of life already equals that found later in the neonatal period. Preliminary observations in children which have been subject to pathological delivery or which have shown clinical signs of cerebral damage or asphyxia, indicate that the adjustments in peripheral circulation to extrauterine life might easily be disturbed and that the circulation, as regards blood flow in the foot and calf, might remain in abnormal states for prolonged periods.

The possibilities of plethysmography in the study of the peripheral circulation in the newborn are manifold. It is also a completely harmless method. Apart from the problems which have only been briefly outlined in the present investigation the study of peripheral blood flow and resistance to circulation offers possibilities of finding out how the cardiovascular system reacts to physiological stimuli, normally, and in various pathological conditions. This will be the subject of future investigation.

Summary

1. Blood flow in the foot and calf has been measured under standard conditions in 15 full-term infants with a water-filled venous occlusion plethysmograph. Technical details and experimental difficulties are discussed.

2. The individual mean flow values varied between 5 and 10 ml/100 ml/min. The mean foot and calf flow during the first day of life was 7.9 ($\pm$ 1.7) ml/100 ml/min. There was no significant difference between these observations and those made later in the neonatal period which had a mean of 7.3 ($\pm$ 1.4) ml/100 ml/min.

3. A large local circulatory reserve could be demonstrated by inducing reactive hyperaemia.

4. The conclusion is made that normal mature infants have attained stability of peripheral circulation in the extremities already during the latter half of their first day of life.

5. Preliminary observations indicate that this stability can easily be disturbed and that children which have been subsubject to pathological delivery can remain in an abnormal circulatory state for long periods.

Acknowledgement

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A Plethysmographic Method for Measuring the Systolic and Diastolic Blood Pressure in Newborn Infants

by OLOV CELANDER and GÖRAN THUNELL

In a previous investigation (4) the possibilities of venous occlusion plethysmography in the study of the changes in circulation at birth have been discussed. This paper describes how observations on the rate of arterial inflow to an extremity can also be used to measure, indirectly, the systolic and diastolic blood pressure in the systemic circulation.

Method

The principles of plethysmography and technical details in the use of this method on infants have already been described elsewhere (4). Fig. 1 shows the underlying principles when observations on blood flow are used to determine the blood pressure. It is quite obvious that, if a sphygmomanometer cuff is inflated at a suprasystolic pressure, the circulation to the limb will be arrested. An arterial inflow will start the very moment the effective cuff pressure falls below the intravascular systolic peaks. This is the basis of the "flush method", suggested by Gärtner (7) already in 1899, but clinically applied only recently (8). The flush method has a sound theoretical background but it is crude and largely subjective. If the rate of blood flow is measured repeatedly at a large variety of occluding pressures in the sphygmomanometer cuff, the effects of the cuff pressure on the rate of arterial inflow can be studied systematically. The highest cuff pressure at which a minimal arterial inflow is recorded must equal the systolic blood pressure. If the observations on blood flow are repeated at successively lower "occluding" pressures, the arterial inflow will show gradual increases, owing to the fact that the perfusing pressure to the extremity will be effective, not only during the systolic peaks, but during increasingly larger fractions of each pulse cycle. A maximal arterial inflow is to be expected once the cuff pressure equals, or falls below, the diastolic blood pressure. An arterial inflow will then occur during the whole length of each pulse cycle. A cuff pressure which is lower than the diastolic arterial blood pressure will not give any further increase of flow. With this method, therefore, the systolic pressure can be defined as the highest cuff pressure which allows a minimal arterial inflow. Likewise, the diastolic pressure equals the highest cuff pressure at which a maximal flow is obtained.

Some modifications in technical details can be made if plethysmography is used only to measure the blood pressure but not the magnitude of blood flow in quantitative terms. It is not necessary to keep strictly constant temperature conditions in the plethysmograph or the room in which the examination takes place. Rapid changes in temperature must, however, be avoided. The plethysmograph does not have to be water-filled. Arterial pulsations in the

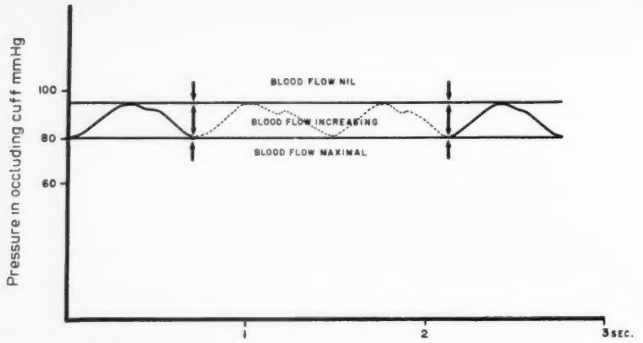

Fig. 1. Underlying principles for measuring systolic and diastolic pressure by plethysmography.

tracings guarantee that there is no lekage of air at the point where the plethysmograph is sealed to the extremity. Calibration of the volume recorder and calculations of the volume of tissue enclosed in the plethysmograph are not required. The cuff pressure at which an arterial inflow commences is easily determined and the pressure at which a maximal inflow occurs can be judged almost by eye from the slopes in the plethysmograms.

In making a suitable sphygmomanometer cuff we have followed the recommendations (11, 14), which were later adopted by the American Heart Association (3). Although differences in weight of the infants and the greater circumference of the leg in comparison to the arm may speak in favour of sphygmomanometer cuffs of different width, uniformity of practice is desirable. We have therefore used a standard width of 2.5 cm for all our observations. It is essential that the inflatable bag of the cuff is long enough to encircle the limb completely. The cuff was made from ordinary adhesive plaster, supported by paper clips, to which a suitable length of rather thin rubber tubing was adhered. It was kept in position by short pieces of adhesive plaster. The tubings for joining the cuff to the pressure reservoir and to the mercury manometer, and also the necessary connectors, were taken from used transfusion sets. Fitted to the baby's limb the cuff had strength to withstand a pressure of 150 mm Hg.

Results

We have used this plethysmographic method in more than 50 infants, either in observations on the blood pressure alone or, more often, in connection with determinations of the blood flow in the foot and calf. Only occasionally did we have to give up, owing to restlessness of the child or because of nursing requirements. The measurements were performed at different stages of the neonatal period and the group of children was rather mixed, including full-term normal infants, but also babies which had been subject to pathological delivery, or which had shown clinical signs of different pathological conditions. In this communication we do not intend to present any detailed data regarding the changes of blood pressure at early neonatal age. We rather wish to illustrate the practical application of our method.

Fig. 2 illustrates the procedure for taking blood-pressure readings. The subject was a three-day-old postmature child, delivered by the vacuum extraction method. No arterial inflow was recorded at cuff pressures exceeding 116 mm Hg. When the cuff was inflated at this pressure (marked by the arrow in the bottom tracing) a

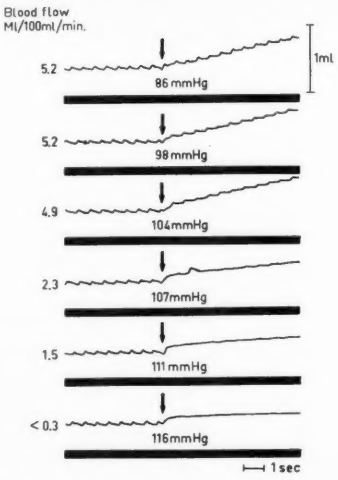

Fig. 2. Blood flow tracings at different cuff pressures. Postmature child delivered by the vacuum extraction method. Three days old. Blood pressure 116/98. Note the absence of arterial pulsations in the two bottom plethysmograms.

minimal arterial inflow, less than 0.3 ml/100 ml of tissue/min, was recorded. Some 20 sec later the observation was repeated at a cuff pressure of 111 mm Hg. As seen from the slopes in the plethysmograms the arterial inflow was then more rapid (1.5 ml/100 ml/min), and further increases of flow were obtained when the cuff pressure was lowered to 107 mm Hg and then to 104 mm Hg. A maximal arterial inflow (5.2 ml/100 ml/min) occurred at a cuff pressure of 98 mm Hg and further lowering of the occluding pressure down to 50 mm Hg did not effect any additional increase of blood flow. As we record the systolic blood pressure as the highest cuff pressure at which a minimal arterial inflow is obtained, and diastolic pressure as the highest cuff pressure allowing maximal arterial inflow, the blood pressure in this child was 116/98 mm Hg. This is the highest pressure that we have yet recorded in the newborn.

Even if this method is primarily based on blood flow determinations the plethysmograph can also be used as a pulse indicator in the sphygmomanometric sense. As seen in Fig. 2, the plethysmograph is sensitive to those small volume changes in the extremity which are effected by the arterial pressure waves. However, the presence of pulsations distal to the occluding cuff is not always an entirely safe indicator of the systolic level. In the two bottom tracings in Fig. 2 arterial pulsations are absent in spite of the fact that the cuff pressure must be below the systolic peaks, as judged from the observations on arterial inflow. The slight irregularities seen in the plethysmograms were synchronous with the respiration of the child but not with the heart rate. Even at a cuff pressure of 107 mm Hg the arterial pulsations were not very prominent and most sphygomanometric methods would therefore, most probably, have given readings of the systolic pressure which were too low. In other subjects arterial inflow and arterial pulsations were interrupted more simultaneously (Fig. 3). When the cuff was inflated at a pressure of 98 mm Hg, which must be very close to the systolic pressure, a displacement artefact was first seen. During the following first few seconds the arterial inflow was minimal and pulsations were absent. Flow and arterial pulsations then both

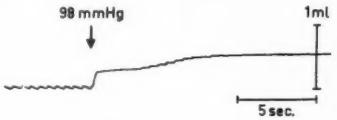

Fig. 3. See text.

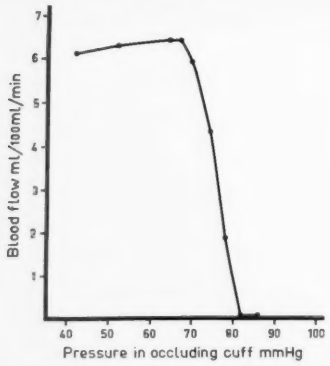

Fig. 4. Diagram showing the relation between the pressure in the occluding cuff and the rate of arterial inflow to the foot and calf in a normal full-term infant two days of age.

reappeared for a short period, only to disappear again. The most likely explanation for this phenomenon is that the rapid inflation of the cuff evoked a short general increase of the blood pressure, owing to the interruption of the circulation to the leg.

In Fig. 4, the rate of blood flow in the foot and calf in a two-day-old normal infant has been plotted against the occluding pressure. At cuff pressures below 68 mm Hg the arterial inflow remained largely unchanged at four separate observations (slightly above 6 ml/100 ml/min). At cuff pressures higher than 68 mm Hg the rate of arterial inflow rapidly declined, and no definite flow occurred at occluding pressures from 82 mm Hg upwards. In this case, the systolic blood pressure fell between the limits of 78 and 82 mm Hg. The diastolic pressure was recorded at 68 mm Hg. This gives a pulse pressure of 10 to 12 mm Hg, which is quite small compared with adults. However, there are good reasons for assuming that the pulse pressure in the newborn and in infants is normally quite small. The explanation for this difference between infants and adults will be discussed below.

Discussion

Recent publications (1, 9, 10, 12) have reviewed the various methods that have been used for measuring the blood pressure in the newborn. The fact that new alternatives are introduced almost every year is suggestive that no method has yet been considered sufficiently accurate and convenient to be generally adopted. With the exception of the "flush-technique", previous methods are all sphygmomanometric, and the systolic blood pressure level is decided by the detection of arterial pulsations distal to the blood pressure cuff or the auscultation of Korotkov's sounds. After the ordinary methods of detecting arterial pulsations in adults by palpation and auscultation had been found unsatisfactory in infants, a number of sensitive pulse indicators were introduced (1, 10, 12). Others picked up Korotkov's sounds by special microphones, the most recent contribution being that by Morse, Brownell & Currens (9). Various indirect methods have been compared for accuracy, but, apart from the studies by Woodbury *et al.* in 1938 and 1939 (11, 14), no direct comparison with intra-arterial pressure has been made. In the present study a definite arterial inflow was not infrequently recorded at cuff pressures which did not permit the transmission of arterial pulsations. In these cases any method which, as an underlying principle, applies the presence of arterial pulsations would appear to produce an error on the low side.

A major advantage with the method that

we suggest is that diastolic pressures can be recorded. This has not been accomplished by previous methods with the possible exception of the refined auscultatory technique introduced by Morse *et al.* (9). They did not make any comparison with direct intra-arterial pressure recordings, and the interpretation of Korotkov's sounds in relation to diastolic pressure must therefore still be left open. Our method has not yet been compared with direct measurements of intra-arterial pressure in infants. In the adult, on the other hand, Celander & Thorén (5) have undertaken such a comparison by measuring hand blood flow by plethysmography and, simultaneously, intra-arterial pressure in the corresponding brachial artery. In these experiments the agreement between the two methods was quite satisfactory. We also intend to perform a similar comparison in infants by pressure recordings from the umbilical artery.

In our blood-pressure determinations, the difference between systolic and diastolic pressure in the individual infant has been surprisingly small, generally around 15 mm Hg and only occasionally greater than 25 mm Hg. This small pulse pressure, compared with adults, is probably related to the higher heart rate in the newborn. For a given cardiac output the stroke volume must be correspondingly smaller in the infant. A contributing factor is probably the highly distensible arterial system of the newborn which will moderate the pressure changes caused by each stroke volume. Dawes *et al.* (6) have shown that in the newborn lamb a sudden closure of the ductus arteriosus causes a striking diminution in the pulse pressure. A large pulse pressure is also a well-known clinical finding in patients with persisting ductus arteriosus. Ashworth & Neligan (2) has recently suggested that "a satisfactory method of measuring the diastolic pressure in babies might be expected to help in elucidating the changes in the functional capacity of the ductus".

Although we consider that our method is fairly accurate in measuring both systolic and diastolic blood pressure in infants, we do not claim that the plethysmographic method, as described here, is suitable for everyday clinical use. If only the systolic pressure is required, there are other less inconvenient methods (1, 9). If, on the other hand, it is found desirable to determine both the systolic and the diastolic pressure, plethysmography will probably be the method of choice. In order to escape the inconvenient and space-consuming recording on a kymograph with smoked paper, we are now working out a simplified version of our method. The plethysmograph has then been substituted by a "mercury-in-rubber" gauge (13) connected to an electromanometer. The pulsatile volume changes of the extremity can be recorded on an ordinary electrocardiograph. Similarly one can record the increase in circumference of the limb which occurs during the short collecting periods when the sphygmomanometer cuff is inflated. In taking a blood-pressure reading it is only necessary to determine the highest cuff pressures at which minimal and maximal arterial inflow is observed. This can be decided from the slopes in the plethysmograms, and if only pressure determinations are intended, it is not necessary to measure blood flow in quantitative terms. Still it must be stressed that measurements of peripheral blood flow in quantitative

terms are worth-while, since blood pressure readings alone give rather limited information as to whether the circulatory state of the child should be considered normal. Combined flow and pressure measurements afford possibilities to determine the local peripheral resistance to circulation. This is one of the main control mechanisms in the circulatory system.

Summary

Plethysmography of the foot and calf has been used for measuring the blood pressure in the newborn. We regard as the systolic level the highest pressure in the occluding cuff at which a minimal arterial inflow can be recorded. Diastolic pressure equals the highest cuff pressure at which a maximal arterial inflow is obtained. The pulse pressure appears to be small in the newborn, in the majority of our cases less than 20 mm Hg. Although our blood-pressure determinations are based on observations of blood flow, the plethysmograph can also be used as a "pulse-indicator", since arterial pulsations are reflected in the tracings. Not infrequently, however, a definite arterial inflow was recorded at cuff pressures which did not transmit any pulsations beyond the occluding cuff. Under such circumstances sphygmomanometric methods would probably have given readings of the systolic pressure which were too low. The pros and cons in the use of this method in clinical work and in circulatory studies are discussed.

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Studies on an Abnormal Hemoglobin Causing Hereditary Congenital Cyanosis

by H. A. HANSEN, O. R. JAGENBURG and B. G. JOHANSSON

In 1942 Thysell (19) described a family with non-cardiac, hereditary cyanosis. Hansen, Jagenburg, & Reinand (7) later showed that the blood of the affected members of this family contained a spectrophotometrically abnormal hemoglobin, which was designated as Norin hemoglobin. The abnormal component differed spectrophotometrically from methemoglobin and reacted neither with cyanide nor fluoride. The oxygen-combining power of the blood was reduced as compared with the iron content and did not increase after administration of ascorbic acid (13) or methylene blue.

Analogous cases of hereditary cyanosis caused by abnormal hemoglobins other than ordinary methemoglobin have been found in another nine families (3, 4, 5, 8, 10, 11, 14, 15). The abnormal hemoglobin described by Hörlein & Weber (10, 11) was designated by Singer (18) as Hb M, as it was assumed to be a methemoglobin. It was recently shown by Gerald (3) that there are several variants of hemoglobin M, and up to now four different types seem to have been described (5, 15).

Using starch-block electrophoresis Gerald (3) succeeded in separating the abnormal hemoglobin M component from the normal hemoglobin. The electrophoretic separation was, however, only satisfactory after previous oxidation of the hemolysate with potassium ferricyanide. Without oxidation a diffuse band was obtained in which three zones could be distinguished on the basis of colour.

Concerning the Norin hemoglobin it has now been possible for us to isolate small amounts of the abnormal component without previous oxidation both by agar gel and moving-boundary electrophoresis. The isolated component has been studied spectrophotometrically. The results of these studies together with the hereditary features of the anomaly will be reported in the following.

Methods

The blood samples were obtained by venipuncture and collected in heparinized flasks. The plasma was removed by centrifugation and the red cells were washed 5 times, each time with four volumes of saline. The hemolysis was achieved by the addition of 2 volumes of distilled water and 0.4 volumes of toluene to the red cell suspension (3). The stroma was removed by high-speed centrifugation at 20,000 *g*. All operations were performed at +4°C.

Agar gel electrophoresis. Agar gels were

prepared from Difco Noble Agar suspended in 0.02 *M* sodium phosphate buffer. Usually a buffer of pH 7.1 was used. The agar concentration in the gels was 1.0 per cent (w/v) and the dimensions of the agar plates were 12 × 14 cm. Before the electrophoretic run, a basin 0.4 × 12 cm was cut out in the gel perpendicular to the running direction and filled with a mixture of one volume of hemolysate and one volume of 1 per cent fluid agar (cooled to +40°C). 1.0–1.5 ml of hemolysate containing about 100 mg of hemoglobin was separated in each run. The electrophoresis was performed at room temperature for 5 hours with a current strength of 35 mA. When blood from the cyanotic subjects was analyzed, two completely separated zones were obtained (see Fig. 2). The fast moving zone (A) was cut out and eluted in 0.02 *M* phosphate buffer at pH 7.1. The zone between the two components was also cut out and discarded. The basin formed was again filled with fluid agar and after solidification a small basin of dimensions 0.4 × 11 cm was cut out just in front of the abnormal component. The basin was filled with the buffer solution as described above. The run was continued and the slow moving component (B) then emerged into the buffer basin. The buffer solution was withdrawn and replaced with fresh solution after 20, 40 and 60 minutes. In this way 4–5 ml of solution containing component B could be collected.

Moving boundary electrophoresis. Electrophoresis was performed in a Tiselius apparatus using standard cells (11 ml). The experiments were run in sodium phosphate buffer, pH 6.8 and ionic strength 0.1. When separation was complete, small samples of the separated materials were withdrawn from the cells by means of a capillary tube for spectrophotometric examination.

Spectrophotometry. Within a few hours after electrophoresis was complete the untreated eluates were examined spectrophotometrically within the wavelength range 380–650 mμ. The hemoglobin components were thereafter treated with different reagents and again examined spectrophotometrically within the range 450–650 mμ.

Reagents were added in order to produce the following derivatives:

Carbonmonoxyhemoglobin obtained by gently bubbling carbon monoxide (from a mixture of sulphuric acid and formic acid) through the eluates for 3 minutes.

Reduced hemoglobin obtained by the addition of 0.1 ml of a saturated solution of sodium dithionite ($Na_2S_2O_4$) to 3 ml of the eluates.

Methemoglobin obtained by the addition of 0.02 ml of a 10 per cent solution of potassium ferricyanide to 3 ml of the eluates.

Methemoglobincyanide obtained by the addition of 0.02 ml of a 5 per cent solution of neutralized sodium cyanide to 3 ml of the oxidized eluates. The non-oxidized eluates were also treated in the same manner.

Methemoglobinfluoride obtained by the addition of 0.05 ml of a saturated solution of sodium fluoride to 3 ml of the oxidized eluates. The non-oxidized eluates were also treated in the same manner.

All samples were thorougly mixed and left for 2 minutes before the spectrophotometric examination.

An automatically recording spectrophotometer (Zeiss, model RPQ 20A) was used throughout the investigation.

Iron determination was performed according to a method described by Polis & Schmukler (16).

The oxygen-combining power of the whole blood was determined according to van Slyke.

Results

Family studies

The pedigree of the family is given in Fig. 1. All subjects are alive except Nos. 1, 3, and 6. Subject 1 died in 1934 of silicosis at the age of 61. Subjects 3 and 6 both died before one year of age, Subject 6 due to severe malformations and Subject 3 from gastroenteritis.

Blood samples were obtained from the living members of the family, and the

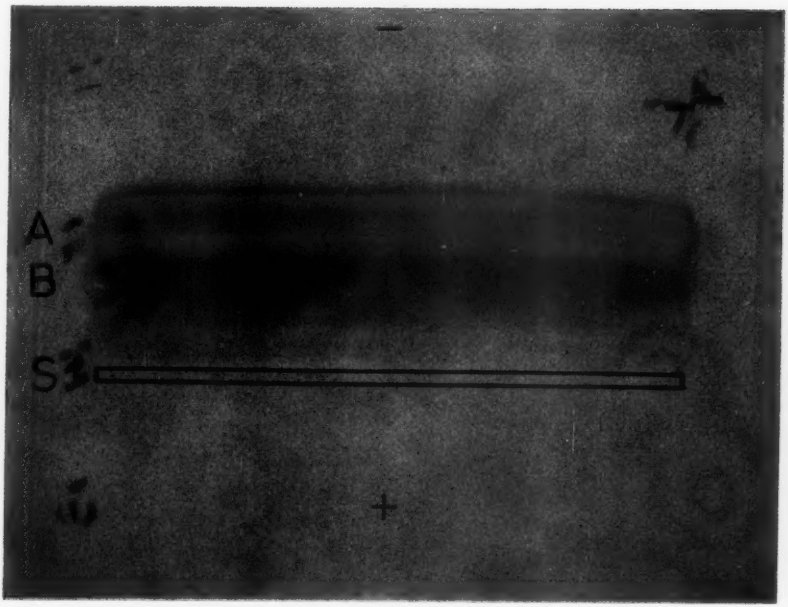

Fig. 2. Agar-gel electrophoretic pattern of untreated hemoglobin from the Norin family. Electrophoresis performed in 0.02 *M* phosphate buffer, pH 7.1. A = normal oxyhemoglobin, B = abnormal component, S = starting basin.

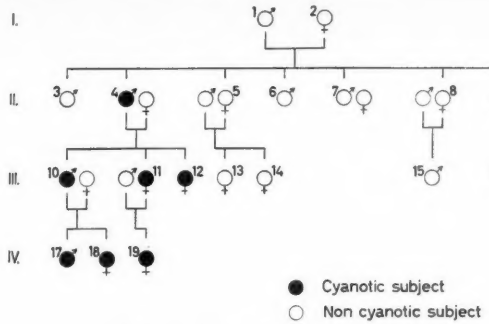

Fig. 1. Pedigree of the cyanotic family.

light-absorption spectra of the hemolyzed whole blood were examined at pH 6.6. The absorption spectra of the hemoglobin obtained from the non-cyanotic members were all identical to that of normal oxyhemoglobin. Blood samples from four of these subjects (2, 7, 8, 9) were analyzed by means of agar-gel electrophoresis. In all instances a single zone was obtained, which showed the same relative mobility and the same bright red colour as normal oxyhemoglobin.

As seen in Fig. 1 there are altogether seven cyanotic members of the family belonging to three generations. They have all been obviously cyanotic since birth, and especially in the newborn infants the cyanosis was very pronounced. They are all apparently healthy and there are no signs of cardiac or pulmonary diseases. For more detailed information about Subjects 4, 10, 11, and 12 the reader is referred to Thysell (19).

From all the cyanotic subjects venous blood was collected and examined on one or more occasions. The freshly drawn blood was always dark brown and the colour was not altered by shaking the blood in the presence of air. The maximal oxygen-combining power of the blood from Subject 4 was 9 per cent less, from subject 11 it was 21 per cent less, and from subject 10 it was 11, 16 and 21 per cent less respectively (determined on three different occasions) than for normal blood with the same iron content.

The light-absorption spectra of hemolyzed whole blood deviated (pH 6.6) in all subjects in a similar way from that of normal oxyhemoglobin. Thus there was an increased optical density between 480 and 520 mμ and between 590 and 670 mμ as compared with normal oxyhemoglobin of the same iron content, whereas the optical density was lower in the region near the absorption maxima at 524 mμ and 577 mμ. The absorption spectra were not significantly changed after the addition of cyanide or fluoride and consequently there was no detectable amount of methemoglobin as determined by the method of Evelyn & Malloy (1).

Blood was collected from Subjects 17, 18 and 19 during their first days of life. The spectrophotometric examinations of these blood samples showed that the abnormal component was present, and that the concentration of this component was

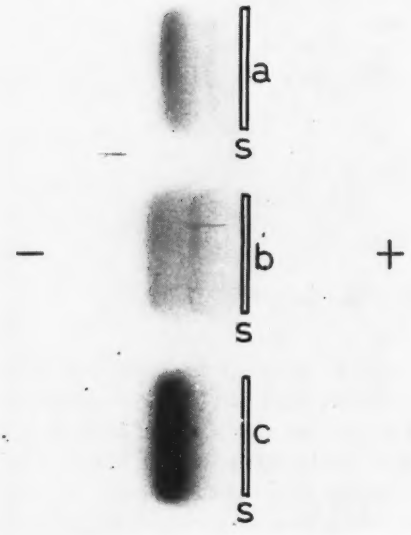

Fig. 3. Agar-gel electrophoretic patterns of hemoglobin from the Norin family. Electrophoresis in 0.02 *M* phosphate buffer, pH 7.1. a = untreated hemolyzate, b = oxidized hemolyzate, c = oxidized hemolyzate treated with cyanide, s = starting basin.

approximately the same as in the older subjects.

The blood samples from Subjects 4, 10 and 12 were also analyzed by means of agar-gel electrophoresis. Similar results as described below were obtained in all three cases.

Isolation of the abnormal component

In the isolation experiments blood samples from Subject 10 were used.

Agar-gel electrophoresis. When untreated hemolysate was analysed, satisfactory separation was obtained at about pH 7. Above pH 7.4 the separation was only partial. Most experiments were carried out at pH 7.1, at which pH two distinctly separated zones were obtained (Fig. 2). Both showed an obvious cathodic migration due to the electroosmotic effect of the agar medium. The fast-moving component (A), which showed the same relative mobility as normal hemoglobin (Hb A), had a bright red colour, whereas the slow-moving component (B) had a typical reddish–brown colour with a tinge of purple.

Normal hemoglobin analyzed under the same conditions gave rise to a single distinct zone.

When the hemolyzed abnormal blood was oxidized with potassium ferricyanide prior to the electrophoresis, two distinctly separated zones were also obtained (Fig. 3), which agrees with the observation of Gerald (3). No separation was obtained when the oxidized hemolysate had been treated with neutralized cyanide before the run.

Moving boundary electrophoresis. As seen in Fig. 4, the two components can be well resolved when run in a phosphate buffer at pH 6.8, ionic strength 0.1. The possibility that the minor peak could be a stationary boundary due to a buffer artefact was ruled out by withdrawing small

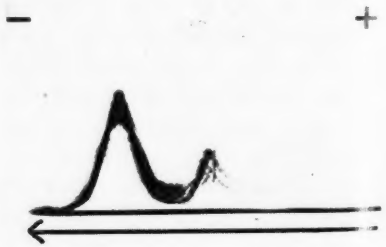

Fig. 4. Moving-boundary electrophoresis of untreated hemoglobin from the Norin family. Phosphate buffer, pH 6.8, ionic strength 0.1. Descending limb after 450 minutes.

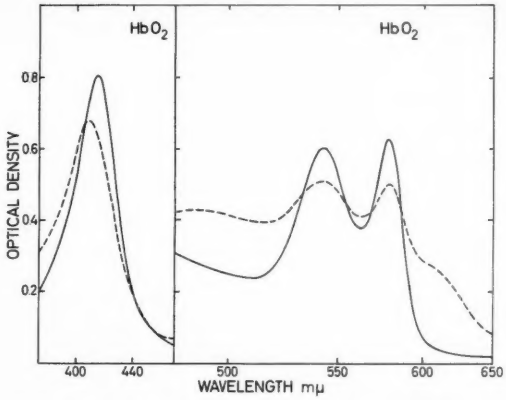

Fig. 5. Absorption spectra at pH 7.1 of the untreated hemoglobin fractions separated by agar-gel electrophoresis. The designations used in this and following figures refer to the normal hemoglobin. Solid line = fraction A (normal hemoglobin). Broken line = fraction B (abnormal hemoglobin). The absorption curves are recorded on solutions with approximately equal iron content.

samples from different regions in the cell and subsequent spectrophotometric examination of these samples. It was found that the material corresponding to the minor peak obviously consisted of the abnormal component, while the major peak had the same light absorption curve as Hb A. The apparent composition of the separated hemoglobin was found to be 86 % Hb A and 14 % of the abnormal component. The major component corresponding to Hb A had a mobility of 0.35×10^{-5} cm^2/volt-sec whereas the abnormal component did not move at this pH.

Spectrophotometry of the separated components

The light-absorption spectrum of fraction A was identical to that which was obtained for normal oxyhemoglobin. The component could be converted to normal carbon monoxyhemoglobin, reduced hemoglobin, methemoglobin, methemoglobin cyanide, and methemoglobin fluoride as judged from the absorption spectra. In describing the spectrophotometric properties of the abnormal hemoglobin, com-

TABLE 1. *Absorption maxima at pH 7.1 of normal hemoglobin and certain of its derivatives as compared with the abnormal hemoglobin component treated in a similar way.*

Hb derivative	Absorption maxima in mμ Fraction A (normal hemoglobin)	Fraction B (abnormal hemoglobin)
Oxyhemoglobin	413	408
	—	490
	542	540
	577	577
	—	600
Carbon monoxyhemoglobin	539	538
	573	573
Reduced hemoglobin	555	555
Methemoglobin	500	495
	630	600
Methemoglobin cyanide	540	533
Methemoglobin fluoride	490	490
	603	603

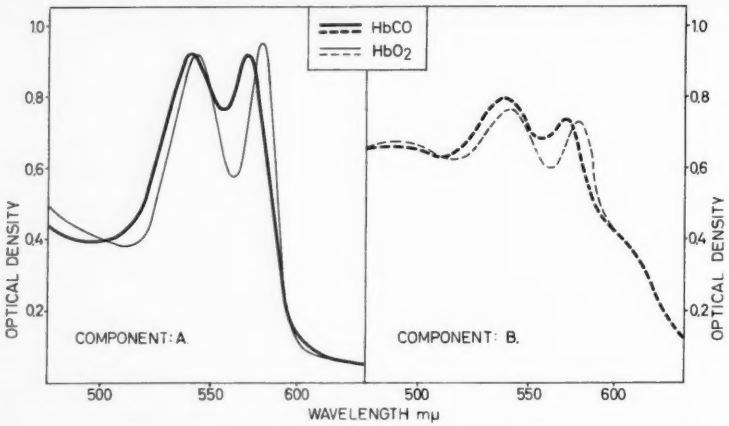

Fig. 6. Absorption spectra at pH 7.1 of the untreated hemoglobin component separated by agar-gel electrophoresis as compared with their carbon-monoxide derivatives. Solid line = fraction A (normal hemoglobin). Broken line = fraction B (abnormal hemoglobin).

ponent A has therefore always been used as a reference in order to illustrate the spectrum of normal hemoglobin.

The absorption spectrum of fraction B deviated obviously from that of fraction A (Fig. 5). In addition to the slight shift of the normal maximum at 542 mμ, there was a marked shift of the Soret band from 413 mμ to 408 mμ. Furthermore, the abnormal component had a maximum at 490 mμ and an increased optical density at about 600 mμ as compared with normal oxyhemoglobin. After addition of cyanide or fluoride neither the normal nor the abnormal component showed any changes in their absorption spectra.

The abnormal fraction apparently had ability to combine with carbon monoxide as there was a shift of the absorption maxima at 540 and 577 mμ towards shorter wavelengths. As seen in Fig. 6 the shifts of the maxima were also accompanied by changes in the optical densities. These changes in the optical densities near the maxima differed for the normal and the abnormal hemoglobin.

When sodium dithionite was added to fraction B, one maximum was obtained at the same wavelength (555 mμ) as the maximum of normal reduced hemoglobin (Fig. 7). The ratio of the optical densities at 555 and 500 mμ was, however, considerably smaller for the abnormal hemoglobin than for normal reduced hemoglobin.

After oxidation with potassium ferricyanide the abnormal hemoglobin gave rise to a compound which spectrophotometrically tallied with methemoglobin M_B (3), but which differed obviously from normal methemoglobin (Fig. 7). At pH 7.1 two absorption maxima were obtained at 495 and 600 mμ, while normal methemoglobin at the same pH has its maxima at 500 and 630 mμ. The ratio of the optical densities at the two maxima (O.D. 495/O.D. 600) was found to be 1.57 for the abnormal methemoglobin, which again is in agreement with the figure

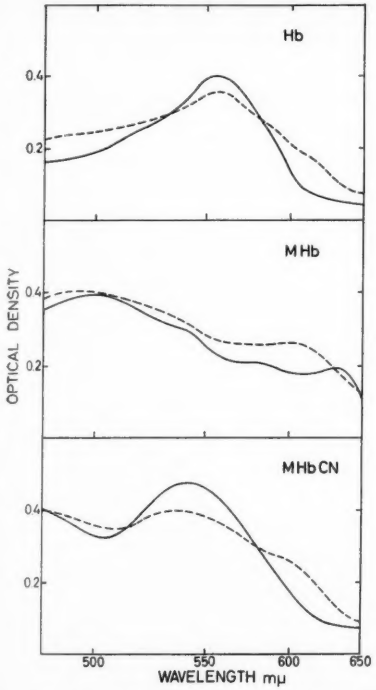

Fig. 7. Absorption spectra at pH 7.1 of certain derivatives (Hb = reduced hemoglobin, MHb = methemoglobin, MHbCN = methemoglobin cyanide) of the hemoglobin components separated by agar-gel electrophoresis. Solid line = fraction A (normal hemoglobin). Broken line = fraction B (abnormal hemoglobin). The iron content of the solutions compared were approximately equal.

given by Gerald for methemoglobin M_B (3). When a solution of the oxidized abnormal globin was compared with a solution of normal methemoglobin with the same iron content and the same pH (7.1), the optical densities of the two solutions were found to be equal at 500 mμ.

The light-absorption spectra of the cyanide and the fluoride complexes of the oxidized abnormal hemoglobin also differed from those of normal methemoglobin. The cyanide complex of the oxidized abnormal hemoglobin (Fig. 7) thus had its absorption maximum at a somewhat shorter wavelength (533 mμ) than normal methemoglobincyanide (540 mμ). Further the ratio of the optical density at this maximum and the minimum at about 500 mμ was lower for the abnormal than for the normal methemoglobin cyanide.

The acid and alkaline hematines obtained from the abnormal component seem to be identical to those obtained from normal hemoglobin as judged from the light-absorption spectra.

Discussion

The abnormal hemoglobin previously designated as Norin hemoglobin (7) is undoubtedly to be classified as a hemoglobin M, a rather heterogenous group of hemoglobins showing obvious differences in their spectroscopic appearance, as compared with other hemoglobins. The designation Norin hemoglobin was chosen on account of the differences in reactivity to the abnormal hemoglobin described by Hörlein & Weber (10, 11) and which was later designated as hemoglobin M by Singer.

The studies of the Norin family indicate that the abnormality is dominantly transmitted probably appearing as a heterozygous character in the cyanotic members of the family. It is noticeable that the oldest generation studied showed no signs of cyanosis. The anomaly could therefore possibly have started as a mutation in Subject 4. This explanation has previously been proposed by Pisciotta *et al.* (15) for the occurrence of hemoglobin M in the two families studied by them. It is also of

interest to note that the members of the Norin family have exhibited a very pronounced cyanosis ever since birth. This is in contrast to the observations of Pisciotta *et al.*, who found a delayed appearance of cyanosis until six weeks to six months after birth. This delay was due, it was suggested, to the presence of fetal hemoglobin. In contrast to Kiese *et al.* (12), but in accordance with Pisciotta (15), we have found a reduced ability of the whole blood to combine with oxygen.

As it seemed to be of fundamental importance to isolate the abnormal hemoglobin without previous oxidation of the hemolyzate, we tried different methods suitable for separation of hemoglobins. Preliminary chromatographic studies on Amberlite IRC-50 (XE 64) (2) and carboxymethyl cellulose (9) showed encouraging results but hitherto the best results have been obtained by means of agar-gel electrophoresis, a method which has been found very suitable for separation of certain other types of hemoglobins (6, 17). Thus a satisfactory separation of the abnormal fraction from normal oxyhemoglobin was achieved at a neutral pH. Using the technique described the difficulties in eluting minor components from the agar gel could be overcome.

The small amounts of separated material obtained have not as yet permitted the application of other methods for studying the homogeneity of the abnormal component. Before it can be considered as a homogeneous compound it must therefore be established that no impurities consisting of, for example, normal oxyhemoglobin occur.

The complexity of the absorption spectrum might be interpreted as due to the presence of the abnormal component partly in an unoxidized and partly in an oxidized form. This does not seem, however, to be consistent with the difference in reactivity to cyanide between the untreated and the oxidized samples of the abnormal component.

As stated by Gerald (5) there are several variants of hemoglobin M differing from each other spectroscopically and in their reactivities to cyanide. A blood sample from a cyanotic member of the Norin family was examined by Dr. Gerald and the abnormal component was found to be indistinguishable from the original Hb M of the Boston type (Hb M_B).[1]

It cannot as yet be considered justified to designate Norin hemoglobin and Hb M_B as abnormal methemoglobins, especially bearing in mind their non-reactivity to cyanide and fluoride. Further studies on the component isolated without previous treatment of the hemolyzed blood may throw light on this problem.

Summary

A study of a family with hereditary cyanosis has been performed. The anomaly was found to be dominantly transmitted and caused by the presence of an abnormal hemoglobin component.

A separation of the unoxidized blood hemolyzate into apparently normal oxyhemoglobin and an abnormal component was achieved both by agar-gel electrophoresis and moving-boundary electrophoresis. The electrophoretically separated abnormal component was characterized by spectrophotometric examination. The com-

[1] We are indebted to Dr. Park S. Gerald, Boston, for this information.

ponent is very similar to Hb M of the Boston type.

The maximal oxygen-combining power of the whole blood was reduced.

Acknowledgements

The skilful technical assistance of Miss Anita Wallin is acknowledged.

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Digestive Studies in Ulcerative Colitis

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In the discussion of the pathogenesis of ulcerative colitis it has been suggested that digestive activity of pancreatic enzymes may be a factor in producing the lesions of the colonic mucosa (8). In experimental studies on dogs Portis, Block & Necheles (9) have thus been able to produce lesions of the colonic mucosa by perfusing the colon with 1–2 per cent trypsin solutions. Lake, Nickel & Andrus (7) transposed in dogs a colonic segment into the duodenum distal to the ampulla of Vater; no damage of the colonic mucosa resulted, however, from this operation. The same authors have determined the enzyme content in the duodenum of patients with ulcerative colitis; both in the fasting state and after stimulation with secretin no significant difference concerning the values of trypsin, amylase and lipase was found between these patients and normal controls. In an investigation of the secretin test in 26 patients with regional ileitis or diffuse jejuno-ileitis, and 39 patients with ulcerative colitis Dreiling (5) found an abnormal pancreatic secretion in about 30 and 10 per cent, respectively, consisting of low secretory volume, low bicarbonate values and/or low amylase values.

Signs of general malnutrition are often seen in patients with ulcerative colitis. No thorough investigation of the absorption capacity of the intestinal tract in this disease has, however, hitherto been performed.

The intubation technique has made possible studies of digestive processes at certain levels of the intestinal tract, and such studies have recently been performed in normal infants and infants with steatorrhea after administration of a test meal containing normal food ingredients (Borgström, Lindquist & Lundh (3)). In the investigation presented here the same technique was used for a study of the enzyme activity and the absorption capacity of the intestinal tract in ulcerative colitis.

Experimental

The experimental series comprised eight children—four normal subjects and four patients with ulcerative colitis. The normal subjects (Cases 1–4) had been cared for in the Clinic for certain other diseases not affecting the gastrointestinal tract; they had never had any chronic digestive troubles, and were all in a good condition at the time of experiment. These subjects were aged 11 to 12 years (Case 1, male, 11 years, wt. 2[illegible] kg; Case 2, male, 11 years, wt. 40 kg; Case 3, female, 12 years, wt. 32 kg; Case 4, male, 12 years, wt. 40 kg). In all the patients with ulcerative colitis (Cases 5–8) the duration of the disease was more than half a year, and the rectoscopy and the X-ray examination

TABLE 1. *Clinical Data of Four Patients with Ulcerative Colitis.*

Case	Sex	Age (years)	Weight (kg)	Duration of disease (years)	X-ray[a] of colon (group)	Severity[b] of disease (arbitrary scale)	Remarks
5	F	6	20	1½	II	+ + +	Complicated by severe hemolytic anemia
6	M	11	37	½	III	+	
7	F	13	37	2	III	+ +	
8	M	15	60	5	II	+ +	

[a] Group I indicates that the process according to the X-ray examination involved only the distal part of the colon. When the changes are classified as Group II, the process was found in most parts of the colon. When the changes were found in the entire colon and in some instances also in the lowest part of the ileum the X-ray findings were termed Group III.

[b] According to the general condition of the patients: + = slight (generally not invalided); + + = medium severity (invalided some part of observation period); + + + = serious (invalided generally at least one year of observation period) (Lagerkrantz 1949).

of the colon were typical of this disease. The age of these patients ranged between 6 and 15 years (see Table 2, which also includes the main clinical data).

Technique of obtaining intestinal specimens. This was mainly the same as that described in another paper (3). A commercial duodenal tube, size Number 9, was used for the experiments. The tube was passed under fluoroscopic control into the site of intestine where the investigation was to be performed, i.e. the distal part of duodenum and/or the first or second loop of jejunum. The patients were all able to eat as usual—the tube was passed through the nose.

Experimental diet. The test meal was based on a formula described by Ahrens *et al.* (1). To this had been added in a low concentration radioiodinated human serum albumin (RIHSA), and as a reference substance polyethylene glycol (PEG), a substance which has the property of being soluble in water, and nevertheless not to be absorbed from the digestive tract. The composition of the test meal is given in Table 2.

Collection of specimens. When the test meal was given at 8.00 a.m., the children had been fasting for about 12 hours. The children received about 250 g of the test meal by mouth from a glass. Fractional specimens were then collected by siphonage for 3–4 hours.

Analysis of the samples. The samples of intestinal content obtained were analysed for PEG (= concentration of test meal), fat, glucose, radioactivity (from the I^{131}-labelled protein of the test meal), enzyme activity (trypsin, amylase and lipase) and colour at 400 mμ (indicating the bile colour) as earlier described (3). From the ratio of PEG to fat, carbohydrate and I^{131}-activity in the test meal, and from the same ratios in the intestinal content it was possible to calculate the percentage absorption of the different food constituents. The values thus obtained refer to a given sample collected during a certain time at a certain level of the intestine.

TABLE 2. *Composition of the test meal.*

Ingredients (g)		Fat (g)	Carbohydrate (g)	Protein (g)
Corn oil	74	74	—	—
Skim milk powder	126	0.5	50	63
Dextrose	144	—	138	—
PEG	5	—	—	—
RIHSA containing 5 μC I^{131}	1	—	—	1
Water	1000	—	—	—
Total	1350	74.5	188	64
Mg per ml		60	150	50

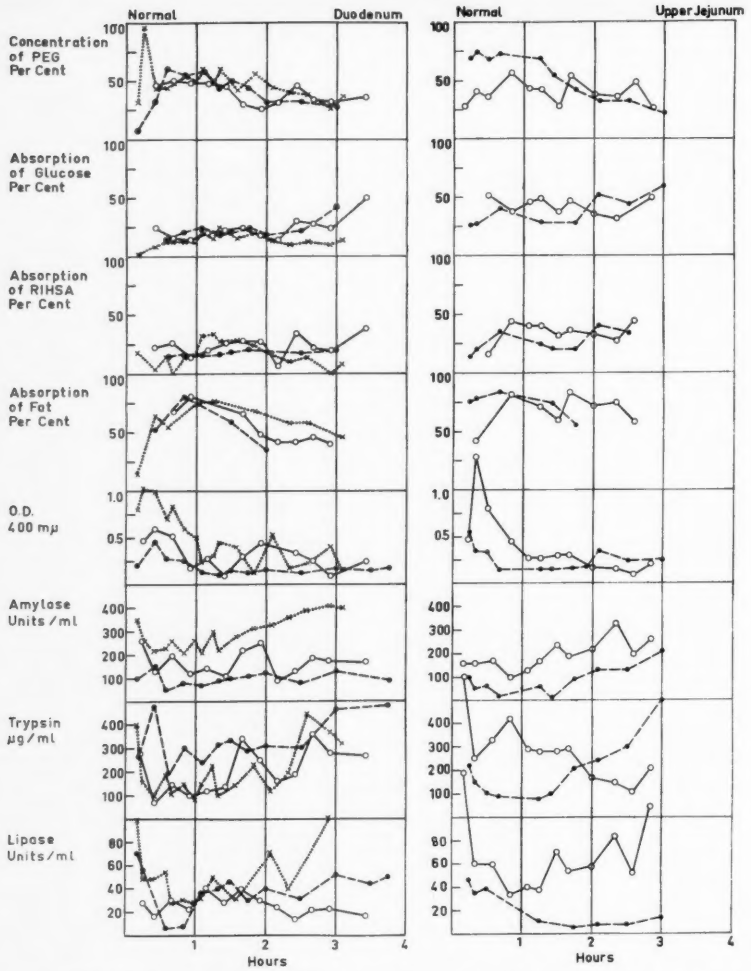

Fig. 1. Data for digestion and absorption processes obtained at the duodenal and upperjejunal level in four normal subjects. *Symbols:* duodenal level, ○—○ Case 1, × ··· × Case 3, ● – – – ● Case 4; upper jejunal level, ○—○ Case 2, ● – – – ● Case 4.

Results

The results are presented in Figs. 1 (normal subjects) and 2 (ulcerative colitis).

Concentration of enzymes in the intestinal content. In the normal subjects as well as in the patients with ulcerative colitis the values for the trypsin concentration proceeded at a level of about 200–300 μg/ml, these values being somewhat higher than those seen in infants (3). In both the experimental groups the amylase concentration varied around 100–200 units/ml during the experimental period, these

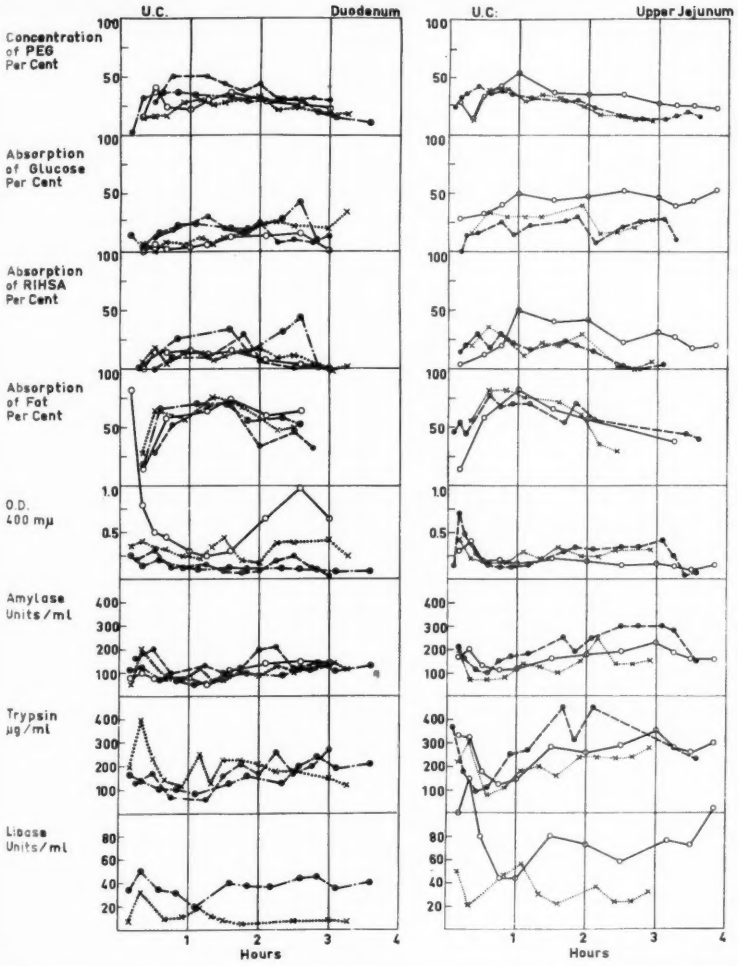

Fig. 2. Data for digestion and absorption processes obtained at the duodenal and upper jejunal level in four patients with ulcerative colitis. *Symbols:* duodenal level, ○—○ Case 5, ●-·-● Case 6, ●– – –● Case 7, × · · · × Case 8; upperjejunal level, ○—○ Case 6, ●– – –● Case 7, × · · · × Case 8.

values being significantly higher than those seen in infants during the first month of life (4). Finally the lipase values varied in both the normal subjects and the patients with ulcerative colitis between approximately 20 and 80 units/ml.

Admixture of bile. The concentration of bile, as indicated by optical density at 400 mμ, varied widely. In one of the patients with ulcerative colitis (Case 5), which was complicated by a hemolytic anemia, excessively high values were

seen; under such conditions no parallelism can, of course, be assumed to exist between the concentration of bile pigments and other bile constituents.

Absorption of food constituents. The concentration of PEG in the intestinal content indicates that in the patients with ulcerative colitis the test meal was diluted about three times when passing the upper intestinal tract, as compared to about twice in the normal subjects; the difference between the two groups is, however, not statistically significant.

In both the experimental groups the values for the absorption of fat varied around 60 and 75 per cent at the duodenal and upper jejunal level, respectively. In the normal subjects the absorption values of RIHSA and glucose were found to be about 20 per cent at the duodenal level, and about 35 and 40 per cent, respectively, at the upper jejunal level. In the patients with ulcerative colitis the corresponding values were at the duodenal level about 15 per cent, and at the upper jejunal level about 25 and 30 per cent, respectively. Thus there was a tendency to a lower absorption of RIHSA and glucose in the patients with ulcerative colitis; the difference is, however, not statistically significant.

Discussion

The results found on the material presented above do not indicate any difference in the secretion of the pancreatic enzymes in patients with ulcerative colitis as compared to normal subjects; in both groups the enzyme curves proceeded at about the same level during the whole experimental period. So far the results of the present investigation do not support the hypothesis that in ulcerative colitis there is an increased digestive activity of pancreatic enzymes on the colonic mucosa.

The absorption of fat, glucose and protein (RIHSA) in the upper part of the intestinal tract was not appreciably affected in the patients with ulcerative colitis as compared with the normal subjects. Thus malnutrition of patients with ulcerative colitis cannot solely be caused by decreased absorption capacity of the intestinal tract; other factors, such as increased loss of endogenous protein from the intestinal tract, are probably also operating.

Summary

The digestion and absorption processes have been studied in four normal children 11 to 12 years of age, and in four patients with ulcerative colitis, 6 to 15 years of age, after ingestion of a well-defined test meal. The upper intestinal tract was intubated and consecutive analysis of the intestinal content obtained by a siphonage were performed.

No significant difference was found between the normal subjects and the patients with ulcerative colitis with respect to the secretion of pancreatic enzymes and the absorption of different foodstuffs in the upper intestinal tract.

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Chronic Pancytopenia with Multiple Congenital Abnormalities (Fanconi's Anaemia)

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In 1927 Fanconi described three brothers who at about the age of 6 years had developed macrocytic, hyperchromic anaemia, which proved fatal within a year of onset. The bone marrow consisted mostly of fat, with scattered haemopoietic foci. These cases were characterized by stunted growth, microcephaly, anomalous skin pigmentation, hypogonadism, strabismus and exaggerated reflexes.

About 90 cases are described to date, in which chronic progressive pancytopenia has been found together with congenital abnormalities. The majority have shown a familial tendency. Estren & Dameshek have reported familial hypoplastic anaemia in childhood, in the absence of other abnormalities. This must be regarded as a variant of Fanconi's syndrome.

The purpose of this article is to review briefly the anaemia of Fanconi, and to present three new cases of the condition.

Distribution

The disease has appeared in various parts of the world, and as well as in Switzerland, it has been described as occurring in several other European countries, in North and South America, South Africa, Turkey and Japan.

The syndrome is seen almost exclusively in childhood: only 4 cases are described in which symptoms have first appeared after the second decade (12, 40, 43). The most usual age of onset is between 4–7 years in boys, and 6–10 years in girls. The youngest victims known were a few months of age (3, 5).

Table 1 shows the sex distribution of 82 cases, and the presence or absence of a familial tendency in these. A further 10 cases appear in publications to which we have not had access.

Symptoms

The increased bleeding tendency is always the most alarming symptom. Affected children have had bruises, severe nose bleeds, and sometimes haemorrhage in the gastro-intestinal tract. The anaemia

TABLE 1. *Published cases of Fanconi's anaemia classified according to sex, and familial or sporadic incidence.*

	♂	♀	Total no.
Familial cases	40	22	62
Sporadic cases	10	10	20
Total no. of cases	50	32	82

has given rise to pallor, fatigue, and even palpitations and breathlessness. Many have been obviously susceptible to infections. A number of cases seem to have arisen in connection with an infective illness.

Haematological Features

The peripheral blood. Pronounced pancytopenia is typical in this syndrome. In exceptional cases the picture has been dominated by, e.g. anaemia (3, 46), leucopenia (37), or combinations such as anaemia with thrombocytopenia (Fanconi's Case 3), or anaemia and leucopenia (van Buchem's Case 2). The fully developed pancytopenia has in some cases preceded by other hypoplastic blood changes, e.g. neutropenia (41), thrombocytopenia (47), anaemia and leucopenia (30). One patient had hypoplasia of the bone marrow without definite changes in the peripheral blood picture (24).

The erythrocytes are typically normo- or hyperchromic, anisocytic, with many macrocytes. Other features have been noted such as poikilocytosis and target cells. Reticulocytes are few in the late stages of the disease, but usually more numerous during the initial stages. Sometimes marked increases with values up to 10–30 % have been noted (16, 29, 35, 46).

The leucopenia consists mainly of a reduction of granulocytes, and there is a relative lymphocytosis, which may amount to 70–80 %.

Very occasionally, features suggestive of increased haemolysis have been noted. Five cases showed increased red cell fragility (5, 23, 43, 49, 50), and 3 showed periodic fever with mild icterus (2, 14, 34). Irregular antibodies have been demonstrated in 1 case, but had probably no pathogenetic significance (42). The erythrocyte life-span has not been determined.

Three cases of leucaemia have been reported in families with Fanconi's anaemia (5, 12, 41). The occurrence of Cooley anaemia trait in another such family was probably coincidental (10) and so was the existence of haemoglobin J in yet another (40).

The bone marrow. Bone marrow seen at autopsy has consisted mainly of yellow fat, with sparse nests of haemopoiesis in certain areas. Marrow smears from living patients have differed widely, partly according to the duration of the disease, and again according to whether the biopsy needle struck a haemopoietic focus or an area of inactive marrow (15). In the early stages erythropoiesis has often appeared active, and has been assessed as absolutely or relatively increased (3, 4, 14, 15, 29, 46). Erythropoiesis appears to be preserved longer than myelo- and thrombopoiesis. In the later stages the bone marrow has shown progressively fewer cells.

A number of authors have observed changes in the reticulo-endothelial structure of the bone marrow (2, 4, 27, 43, 46), consisting of increased fibrosis, and an abnormal preponderance of reticular cells and plasma cells, sometimes even mastocytes. Rohr interpreted these bone-marrow findings as the primary changes characteristic of the syndrome.

Higashi *et al.* demonstrated in their case a weak peroxidase reaction in the bone marrow, and even in the red cells when the anaemia was advanced.

The active type of haemopoiesis in bone marrow, together with the reticulo-

TABLE 2. *Different abnormalities in 68 published cases.*

Abnormal pigmentation	51
Skeletal deformities	40
Stunted growth	38
Small head	29
Renal anomalies	19
Strabismus	18
Microphthalmia	9
Ptosis	2
Epicanthus	2
Nystagmus	2
Deafness	7
Deformities of the ear	3
Exaggerated reflexes	9
Mental backwardness	14
Hypogenitalism	15
Cryptorchism	5
Hypospadias	3
Transposition of penis and scrotum	1
Adiposity	5
Cardiac murmurs	19
Congenital heart disease	4

cytosis, is more suggestive of a haemolytic condition than of chronic panmyelophtisis. The discrepancy between the severe anaemia and the active-looking bone marrow has been taken as indicating that the bone marrow, though markedly reduced quantitatively, is still qualitatively normal, and capable of reactive hyperplasia (15, 35, 46). Certain writers have pointed out a suppression of maturity in the bone marrow (2, 3, 5, 41, 43), but this may be due to an increase in production with release of young forms into the circulation. In all probability, however, there are qualitative changes in the marrow, even in the active foci. Evidence in favour of this is given by the demonstrated changes in the reticuloendothelial structure of the marrow, the discrepancy between erythropoietic activity and myelo- and thrombopoiesis, and the progressive course of these bone-marrow processes.

TABLE 3. *Different skeletal deformities in 40 cases of Fanconi's anaemia.*

Thumb deformities	28
Reduced number of ossification centres in the wrist	17
Aplasia of 1st metacarpal	7
Hypoplasia of 1st metacarpal	2
Aplasia of radius	7
Hypoplasia of radius	3
Absence of lower arm	1
Broad base in proximal phalanges	3
Congenital dislocation of the hip	3
Syndactylism	3
Sprengel's deformity	1
Scoliosis	1
Cervical rib	1
Hip disease of Perthe's type	1
Club foot	1
Increased impressiones digitatae in cranium	4
Generalised osteoporosis	5

Associated Anomalies and Malformations

Among the 82 reviewed cases of Fanconi's anaemia, there were 13 who only had pancytopenia without malformations or constitutional anomalies (1, 8, 10, 14, 19). One case was not admitted because of lack of information. The various findings in the other 68 cases are represented in Tables 2–4. The most common changes are abnormal pigmentation, stunted growth, and deformations of the distal radial portions of the upper limbs. Features quite often seen are abnormally small head, strabismus, malformations of the kidney, and small genitalia. About one fifth of the patients are mentally retarded.

TABLE 4. *Various renal deformities in 19 cases of Fanconi's anaemia.*

Renal aplasia	10
Double pelvis and/or ureters	5
Horseshoe kidney	3
Hydronephrosis	2
Abnormally low situation of kidney	5
Congenital renal cyst	1

Increased pigmentation is seen mostly on the trunk, often perianogenitally, in the groins and axillae, and round the neck. It may appear as mottling, or as large patches with diffuse boundaries, within which smaller patches of viteligo type may occasionally be seen (12, 36, 47, 50). The pigment is melanin (5, 21, 33). Pigmentation may take its appearance before the blood changes are manifest (5, 16, 21, 22), at the same time (20), or at a later stage. The cause of this increased melanin deposition is unknown.

It seems remarkable that deformities are seen so often in the upper limbs, and so seldom in the lower limbs (Table 3). The distal radial structures are particularly affected: thumb, 1st metacarpal, radial wrist bones, and the radius. The reduced number of ossification centres in the wrist has sometimes been interpreted as delayed ossification (5, 21, 36), or a lowered bone age (13, 32, 42). In an orthopaedic series of patients with radial defects (8) a reduction was also noted in the number of ossification centres in the wrist, and was explained here by the fact that the wrist bones were missing or rudimentary, or that individual centres of ossification had merged to form larger bones. In a few patients with Fanconi's anaemia similar changes have been demonstrated (2, 9, 20), which suggests that the reduction in number of ossification centres is a question of malformation.

Tendency to absence or merging of organ embryos is seen again in the urinary tract, where aplasia of the kidney is the commonest anomaly. There is a significantly high incidence of horse-shoe kidney (2, 4, 18). In 5 of 10 cases of solitary kidney, the latter was found to have double pelvis and/or double ureters (12, 34, 36, 41, 47). In these cases the solitary kidney may be the result of merging of the bilateral embryonic organs; evidence for this is provided by the following two observations. The case reported by Cowdell *et al* was noted to have a single kidney, but two renal arteries and two ureters which opened one into each side of the bladder. van Buchem *et al.* found an ectopic left kidney lying on the right side, below the right kidney, in 2 siblings.

In 15 boys the genitalia were described as small, some as very small (21, 30, 47). It is difficult to accept this as evidence of hypogonadism in patients below the age of puberty. One classes this finding rather, along with short stature, little head, and small eyes, as a sign of hypoevolution. In 2 of the 4 adult males, however, clinical signs of hypogonadism were found (12, 43). An abnormally small hypophysis was found at autopsy in 2 cases (12, 32). Adrenal hypoplasia has also been described (36).

Obesity has been noted in 2 boys (49) and 3 girls (3, 20, 46). The girls had enlarged breasts, but no other sign of early sexual maturity.

Cardiac murmurs have commonly been discovered, but definite cardiac malformations reported in only 3 cases (12, 20, 34). A number of others had been assessed clinically as certainly having heart lesions, on the grounds of cardiac enlargment together with type of murmur, but in these no abnormality could be demonstrated at autopsy (21, 35, 47). The murmurs are probably in most cases of anaemic origin.

Treatment

Iron, liver extracts, vitamin B12, folic acid, testosterone (12), ACTH and cortisone have all been tried in treatment, but without effect. The patients have tolerated their low blood values relatively well, but sooner or later have come to require repeated blood transfusions.

Splenectomy was carried out in 19 cases, of which 2 died in the postoperative period. In 8 of the others there was no improvement. Four cases showed a temporary improvement (3, 22, 28, 43). The need of blood transfusions diminished in 2 cases (9, 20). Enduring benefit was noted in 3 cases (14, 15, 19). One of these was atypical and the improvement cannot with certainty be attributed to operation (14).

Resection of spleen should only be undertaken where the bone marrow retains the power of active regeneration (19, 22). A quite small increase in leucocytes and thrombocytes can in particular cases reduce the risk of severe infections or haemorrhage (28). Francis *et al.* maintain that, with regard to the chronic, progressive nature of the disease, any effect of splenectomy can at best be temporary, and suggest that repeated blood transfusions are the only feasible treatment.

Clinical Course and Prognosis

The course of the disease is typically a slow progressive one. The majority of recorded cases have terminated fatally. Time of survival of those dead was in many cases 2 years or less from the appearance of symptoms, and in no case longer than 5 years. Of those still living at the time of publication 5 had survived for more than 5 years (3, 9, 15, 16, 39). The longest survival reported was 15 years (3) but this case was not typical, and the diagnosis perhaps uncertain. In exceptional cases the progressivity of the disease seems to disappear, at least for long periods (3, 15, 19).

The most usual causes of death have been severe anaemia, haemorrhage occurring intra-cranially or into pericardium or gastro-intestinal tract, and infections.

Genetic Considerations

Most cases of the disease have a familial background which indicates a genetic origin. In 3 sporadic cases there was a history of infection in the mother during the first months of pregnancy (2, 20, 46). There are no other known environmental factors. In a pair of binovular twins, one twin had the disease while the other was healthy (19). In almost all cases where the birth weight was recorded, it was lower than normal without the child being premature: this must be regarded as evidence of deranged development already *in utero*.

The 62 cases with a familial background were distributed among 30 families. Ten families presented only one case each of Fanconi's anaemia, these being regarded as familial because of consanguinity in the parents (6, 17, 25, 32), the occurrence of similar abnormalities (11, 12, 27, 32, 34) and/or other blood dyscrasias than pancytopenia (5, 12, 23) in siblings or other close relatives. An illustrative example is given by Cowdell *et al.*, who described 2 brothers. These were both of small stature, with abnormal skin pigmentation, deformities of the hands, and small genitalia.

TABLE 5. *The occurrence of Fanconi's anaemia, congenital abnormalities without evident blood disease, and mortality of other origin among 125 children in 22 families with familial cases of Fanconi's anaemia.*

	No. of cases
Fanconi's anaemia	47
Congenital defects[a]	11
Perinatal mortality	11
Mortality from infectious disease	3
Healthy individuals	54

[a] One case died aged 3 days, further two before reaching 18 months. Leukaemia appeared in one case.

One brother had a typical Fanconi anaemia, and the other an undifferentiated leukaemia.

The familial cases nearly all occurred in siblings. Blood changes in the parental generation were noted in only 2 families: slight pancytopenia in the mother (13), and in the other instance, neutropenia in the father, paternal grandmother and the father's uncle (23). In 2 other families there was a history of tendency to epistaxis in the elder generation (19, 21). Malformations of similar type in both parents and children were only seen in Imerslund's case (32).

Since the disease is nearly always fatal in childhood, there is little possibility of reproduction among these patients. In the family described by van Buchem *et al.* there were two sisters with typical Fanconi anaemia, and a third sister with similar deformities and bone-marrow changes, but only a mild thrombocytopenia. The latter sister gave birth to a child with microcephaly and meningocele, which survived only a few days. Nothing more is recorded concerning offspring to patients with Fanconi's anaemia.

There was consanguinity between parents in 6 affected families (6, 16, 17, 25, 29, 32). Five of these were first-cousin marriages, one a second-cousin marriage.

The genetic defect shows itself in different ways. In a number of cases changes have occurred only in the blood and the bone marrow (1, 8, 10, 14, 19) or the blood changes are combined with only slight congenital anomalies (16, 23, 43). In other cases there had been congenital anomalies without evident blood changes (11, 23, 30, 32, 34, 41) (Table 5). Congenital malformations with slight (9, 16) or quite different (12) blood changes are a rare variant.

The pattern of heredity has generally been regarded as autosomal recessive (6, 41). Imerslund described similar deformities in both mother and child, and suggested the possibility of a dominant heredity. Weicker has calculated that the incidence of illness among younger siblings of Fanconi cases is greater than one could expect, if the gene were recessive.

The following points are of interest: (1) the sex distribution (50 male, 32 female), (2) the high incidence of consanguinity (6 of 30 families), (3) the high incidence of illness among siblings (Table 5), (4) it was exceptional to find parents affected.

There is nothing pointing to a sex-linked heredity. The difference between the incidence of sex is not greater than can be expected by chance. Factors (2) and (4) agree well with the presumption of an autosomal recessive gene. The high incidence of illness among siblings may be an effect of sampling material from literature. The probability is greater that families

with a number of sick children will be reported than isolated cases. The assumption of autosomal recessive heredity is with all probability correct.

It has also been noted in "idiopathic aplastic anaemia" that blood anomalies occurred among relatives with a greater frequency than could be considered as purely coincidental (1, 31).

Radial deformities, which seldom show a familial incidence and are usually explained as early injury or embryonic deficiency, are often accompanied by other abnormalities of the type seen in Fanconi's anaemia (8). Some sporadic cases of Fanconi's anaemia may have the same etiology, but a genetic origin cannot be ruled out, even if the mother has had an infectious disease in the beginning of pregnancy.

A similar syndrome to Fanconi's anaemia is that of congenital hypoplastic thrombocytopenia together with deformities. These patients differ from Fanconi cases in that their symptoms usually develop shortly after birth, and in patients who survive the first year of life the disease is not progressive. The syndrome usually occurs sporadically and has been thought to be the result of early embryonic insult (7, 51). The condition has, however, recently been described in siblings of two families (26, 45) which supplies evidence for a genetic cause even in these cases.

Clinical Histories

Case 1. U.N., born 9.1.1950, referred to the Children's Hospital, Gothenburg, at 7 years of age for investigation of blood dyscrasia. He had a long history of being easily bruised: blood examination had revealed a marked anaemia and thrombocytopenia.

He was No. 3 in a sibship of three; parents and siblings healthy; no consanguinity. Mother's brother had died of sepsis at the age of 9 months. There were no known deformities or blood disease in the family. Pregnancy normal and full-time; delivery normal with birthweight 2700 g.

During the first year of life, he had repeated upper respiratory infections, with admission to hospital on two occasions. Hb had then varied between 8.3 and 9.6 g %. Iron administration had had no effect. At 6 years, he was again admitted to hospital for investigation of anaemia and susceptibility to infection. Hb concentration was then 9.1 g %, R.b.c. 4.0 millions/mm³, and W.b.c. 3700; serum Fe was 137 gamma %.

On examination: A slightly built boy, short for his age (height 118 cm, weight 18.1 kg) and with small head (circumference 47.5 cm). Skin pale, with a number of ecchymoses of recent and older date. Thumbs were deformed and rudimentary, with limited movement in the interphalangeal joint: the left thumb was loosely connected with the rest of the hand. The eyeballs were small, and in the inner corner of the right eye a small epicanthus was noted; there was also a slight convergent strabismus. No abnormality was discovered in heart or lungs. No liver, spleen, or lymph gland enlargement. Testicles were small and firm: the penis small for his age. Neurological status appeared normal. The boy was active and of average intelligence. Fig. 1 is a picture of the patient at 8½ years of age.

X-ray examinations. Skeletal X-rays showed hypoplasia of metacarpal and phalanges of the 1st digit. On the left, the 1st metacarpal was defective proximally. Two wrist bones, most probably os capitatum and os hamatum, were fused in the left wrist, to a single larger bone. There was no osteoporosis. X-rays of heart and lungs were normal. In a straight abdominal X-ray the kidneys appeared to be of normal size and shape: no splenic enlargement was noted.

Laboratory investigations. Haemoglobin level on admission was 7.3 g %, R.b.c. 2.6 mill./mm³, W.b.c. 2000 of which 78 % were

Fig. 1. Case 1 at 8½ yrs of age. Note the rudimentary thumbs.

lymphocytes. Thrombocytes 4000, coagulation time 7 minutes, bleeding time 6 minutes. Prothrombin index 81. The mean red cell diameter was 7.4–7.8 μ. Reticulocyte count 1.5 %. Coomb's direct test was negative. No cold agglutinins or other atypical agglutinins were demonstrable. Serum bilirubin 0.19 mg %. Serum Fe 137 gamma %.

Bone-marrow examination revealed increased deposition of fat. Erythropoiesis was increased relative to other types of cell formation, but an absolute increase was more difficult to demonstrate. Erythropoiesis was normoblastic, but a few macroblasts were seen. In a very few erythroblasts "Jolly bodies" were identified. The red cells showed increased anisocytosis, polychromasia, and a suggestion of poikilocytosis. On special staining to demonstrate the presence of iron, 88 % were seen to be sideroblasts and free iron was plentiful. There was marked reduction in myelopoiesis, though the proportion of mature types was normal: there was moderate toxic granulation. There was an apparent increase in lymphocytes. Megakaryocytes were sparse, immature and not productive of thrombocytes. No thrombocyte agglutinates were demonstrated. Reticular cells were noted in normal numbers. There was also a number of small fibroreticular nests (H. Hansen).

On examination of gastric juice, free hydrochloric acid was found to be present.

The urinary sediment contained agglomerations of red blood cells.

Treatment and course. Iron therapy had previously been tried without success. The boy was treated by repeated blood transfusions, often accompanied by severe febrile reactions. Despite his low blood counts (Table 6) he was very active and took part in sports, though troubled by breathlessness and palpitations. At times he was excessively fatigued, and might have persistent bleeding from nose, gums, or in the skin. Sometimes he had haematuria. The skin of inner thighs and perineum became gradually discoloured slate-grey, and isolated patches of brownish-grey pigment appeared on the trunk.

Marrow examination at a later date showed sparser cells, but a smilar picture in other respects. The mean red cell diameter had increased to 7.8–8.2 μ.

The boy died at home at the age of 9 years, 4 months. The cause of death was probably grave anaemia. Autopsy was not carried out.

Case 2. M.O., a girl nearly 7 years old, born 24.12.1952, first admitted to the Children's Hospital at 2 years of age on account of subcutaneous haemorrhages. Parents healthy and not related. The patient had two brothers, one aged 9, healthy, the

TABLE 6. *Blood values in Case 1, who was born 9/2/1950. About 70–85 % of the white cells were lymphocytes.*

Date	Haemoglobin g %	R.b.c. mill./cumm	Retic. %	Leukoc. cumm.	Thrombocytes
13/2/1951	9.4	3.9		6400	
18/12/1952	9.4			6600	
7/3/1956	9.1	4.0		3700	
13/4/1957	7.3	2.6	1.5	2200	4,000
2/9/1957	7.7	2.5	2.0	3000	16,000
12/11/1957	5.6	2.0	0.8	2400	25,000
10/1/1958	5.9	1.7	3.3	1100	5,000
13/3/1958	5.4	1.8	1.3	2100	11,000
29/7/1958	5.2	1.1	2.5	1600	5,000
4/11/1958	4.6	1.4	1.8	1800	6,000
20/4/1959	1.2	475.000		3400	16,000

other aged 3 being Case 3 described below. Otherwise there was no family history of blood disease. Pregnancy and delivery were normal: birth weight 3170 g.

On examination at the age of 2 the child had been pale, and had a number of skin haemorrhages of recent and older origin. Hb 8.5 g %, R.b.c. 2.8 millions, W.b.c. 6100 of which 84 % were lymphocytes; thrombocytes 12,000. Bleeding time was 7 min, clotting time 5 min. Prothrombin index 90. Clot retraction gave variable results: 34 and 48 % respectively. Tourniquet test for decrease in capillary resistance was positive.

The bone marrow showed cell sparsity, with lymphocytes dominating. Erythropoiesis was increased in relation to myelopoiesis. A number of macroblasts were seen, but no megaloblasts. The erythrocytes showed aniso- and macrocytosis. Myelopoiesis was depressed. No megakaryocytes were seen.

The findings were then interpreted as thrombocytopenic purpura. ACTH and cortisone treatment were tried without effect.

During the ensuing years the child's condition remained largely stationary. She had no pronounced fatigue, and the tendency to easy bruising seemed even to diminish.

She was admitted again at the age of 6 for clinical assessment prior to dental extractions. She was pale, with haemorrhages in the skin, mostly of older origin. She was 117 cm tall, and weighed 20.3 kg. She was of normal build with no evident deformities. A distinct systolic murmur was heard precordially. Liver, spleen and lymph glands were not enlarged. Peripheral reflexes were normal.

Laboratory investigations. The blood values are depicted in Table 7. The red cell picture was characterized by moderate anisocytosis, and a certain degree of macrocytosis. The mean cell diameter according to the Price-Jones curve was 7.3 μ. MCHb 36 $\mu\mu$. Bleeding time was 35 min. Clotting time 7½ min. Serum Fe 150 gamma %. B12 in serum was 525 μg/ml. 5-hydroxytryptamin excretion was 2.8 mg % in the 24-hr urine specimen. There was normal excretion of amino-acids

TABLE 7. *Blood values in Case 2, M. O., born 24/12/1954. About 65–85% of the white cells were lymphocytes.*

Date	Hb g %	R.b.c. mil l/ cumm.	W.b.c. cumm.	Thrombo./ cumm.	Retic. %
1954	8.5	2.6	6100	12,000	
11/1958	8.0	2.2	4300	14,000	1.8
3/1959	5.5	1.8	4300	4,000	
5/1959	6.3	1.8	4500	20,000	3.6
9/1959	6.0	1.3	4000	14,000	2.6

and other ninhydrin-positive substances in the urine (R. Jagenburg).

The bone marrow showed cell sparsity with markedly depressed myelo- and thrombopoiesis. Erythropoiesis was relatively well preserved. There was no apparent increase in fat deposition or fibro-reticular structures (H. Hansen).

Case 3. H.O., younger brother of Case 2, born 7.1.1956. Had always shown a tendency to easy bruising. He was noted to have isolated small haemorrhages in the skin, but general examination was otherwise negative. At the age of $3\frac{1}{2}$ his height was 103.5 cm.

Laboratory values. Hb 11.0 g %. Erythrocytes 3.4 millions, leucocytes 3800, of which 68 % were lymphocytes; reticulocytes 1.3 % and thrombocytes 19,000. Bleeding time was 2 min 15 sec. Clotting time $4\frac{1}{2}$–5 min. The red cells showed slight anisocytosis, and their mean diameter was 7.3 μ.

Comments

Case 1 is a typical example of Fanconi's anaemia. The boy was short in stature, with a small head, small eyes, small genitalia, rudimentary thumbs and 1st metacarpals, strabismus, and subsequent appearance of abnormal skin pigmentation. He had a marked pancytopenia which proved to be progressive. Erythropoiesis in the bone marrow appeared to be active. It was possible that his mother's brother had had the same blood disease.

Case 2 and 3 were siblings. They showed no other abnormality than the blood changes. The peripheral blood picture in Case 2, as well as the bone marrow's appearance, corresponded with those of Case 1. The younger brother had a pronounced thrombocytopenia, but little more than a suggestion of anaemia and leucopenia. These two, brother and sister, must be regarded as examples of Estren & Dameshek's variant of Fanconi anaemia.

Splenectomy was suggested, and declined by the parents of Case 1. It is conceivable that splenectomy should be attempted in Case 2. As long as the condition remains stationary, however, we are unwilling to take the risks of splenectomy, especially since the results of operation cannot be predicted.

Summary

A review of Fanconi's anaemia is given, based on 82 published cases. The disease is characterized by chronic pancytopenia in combination with other abnormalities such as abnormal pigmentation, stunted growth, deformities of upper limb, small genitalia, and kidney anomalies. A variant of the syndrome is familial pancytopenia without deformities. Symptoms of blood dyscrasia are noted in childhood, and there is usually progress to a fatal issue within a few years.

In most cases the disease shows a familial background. The pattern of heredity is with all probability autosomal recessive.

A typical case of Fanconi's anaemia is presented. In addition a brother and sister are described, with blood changes in the absence of other abnormalities.

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Is the Rate of Hypertrophic Pyloric Stenosis Declining?

by ARVID WALLGREN

Introduction

In 1941 the author published an account of his experience of the incidence of hypertrophic pyloric stenosis in Gothenburg during the period 1934–1940. It was stressed that the conditions for estimation of the true frequency of the disease were exceptionally satisfactory in Gothenburg. The diagnosis of infantile pyloric stenosis had been one of the chief interests not only of the pediatricians at the Children's Hospital but also of the few practising pediatricians in the city. The Children's Hospital was the only one for sick children in Gothenburg and all infants in need of hospital treatment were referred to this institution. The Infant Welfare Centres were conducted by the pediatricians and were attended by 80 % of infants in Gothenburg. If there was a suspect case of hypertrophic pyloric stenosis among the attending infants, the child was sent to the Hospital for X-ray examination or observation. In this way it was concluded that although the recorded number of cases of hypertrophic pyloric stenosiss must be considered as minimal, only exceptionally did a manifest case of the disease escape detection. The altogether symptom-free cases could, of course, not be included.

The incidence of hypertrophic pyloric stenosis was in the 7-year period mentioned estimated to be 4 per 1000 live infants with annual variations between 2.5 and 5.7 per 1000. This study was a first attempt at determining the actual incidence of hypertrophic pyloric stenosis in a community. It was emphasized that the observed incidence might serve as a basis for comparison with the figures from the estimated incidence of the disease in other countries.

Since the publication of this study knowledge of the incidence of hypertrophic pyloric stenosis in various countries has improved with data from several places. Although the conditions for not missing any case were not as favorable as in Gothenburg, certainly not many manifest cases have been omitted, and thus a comparison of the incidence may, with some reservation, be made.

Dawson in 1946 reported the incidence in Newcastle-upon-Tyne during the period 1939–1942 to be 2.8 per 1000 live births and maintained that there is no significant difference between this figure and the Gothenburg figure. In 1951 Lawson (5) showed the figure for the incidence in Dundee during the period 1940–1945 to be 1.5 per 1000 live births and stressed that this is a significantly lower incidence

as compared with the figures from Gothenburg. In Edinburgh for the period 1950–1958 Smith (6), in 1960, reported 3.6 per 1000 live births and in Birmingham the incidence was estimated to be 3.10 per 1000 live births, a figure given by McMahon, Record & McKeown in 1951.

In 1941 the present author quoted some published and personal communications with regard to the non-occurrence of pyloric stenosis in the population of other parts of Europe (Turkey) and in tropical countries (Beligan Congo). We have now obtained information that the disease may occur even in the African race (Petcher; Luder; Hamilton; Arya & Hall-Craggs (2)) but only single cases of the disease have so far been reported and we do not know how low the incidence of the disease in this part of the world is. That there must be a very significant difference between the incidence in Africa and in Sweden and Great Britain seems obvious. One must, however, keep in mind the reluctance of the African mothers to take their infants to physicians and the superstition that morbid symptoms depend on the wrath of the gods and the parents' tendency to rely upon witch-craft instead of Western medicine for the treatment of ailments. In the sum total it is quite impossible at present to obtain reliable knowledge of the number of live births and the number of cases of hypertrophic pyloric stenosis in these underdeveloped countries.

When the author moved from the Children's Hospital in Gothenburg to the Pediatric Department of Norrtull's Hospital in Stockholm in 1942 he was struck by the small number of cases of hypertrophic pyloric stenosis hospitalized at the latter hospital as compared with the cases treated in Gothenburg. This impression was further enforced by the author's subsequent local experience and by personal interviews with pediatricians in Stockholm regarding the incidence of pyloric stenosis at present and previously. They felt that the incidence had decreased very substantially. Since the very suitable prerequisite conditions for determining the exact incidence of infantile pyloric stenosis in Gothenburg did not exist in Stockholm, the impression of a progressive declining rate in Stockholm might be solely apparent, rather than real. In order to study the development of the incidence of hypertrophic pyloric stenosis, the author therefore again focused his interest to the conditions in Gothenburg, thus fulfilling the suggestion put forward in 1941, viz.: "... if an investigation similar to the present one is carried out twenty to thirty years hence, it will be possible to obtain definite information with regard to the incidence of the disease, that is, whether it is increasing, decreasing or remaining stationary."

Material and Method

A visit to Gothenburg gave evidence that the prerequisite conditions for obtaining sufficient reliable data regarding the number of cases of manifest pyloric stenosis were almost as satisfactory as in the 1930's. Thanks to the invaluable help rendered the study by Professor Rolf Zetterström, the present Head of the Pediatric Department of the Gothenburg Children's Hospital and Doctor Erik Gedda, the Head of the Children's Welfare Centres in Gothenburg, the project to

TABLE 1. *Incidence of hypertrophic pyloric stenosis among children born in Gothenburg from 1934 to 1940, and from 1950 to 1959.*

Year	No. of infants born alive	No. of infants with pyl. sten.	Incidence of pyl. sten. per 1000
1934	2992	17	5.7
1935	3147	13	4.1
1936	3347	13	3.9
1937	3643	9	2.5
1938	4037	13	3.2
1939	4288	15	3.5
1940	4188	22	5.3
1934–1940	25,642	102	4.0
1950	5774	6	1.04
1951	5562	9	1.62
1952	5463	14	2.55
1953	5686	14	2.46
1954	5653	8	1.41
1955	5807	14	2.40
1956	5947	15	2.53
1957	6236	15	2.40
1958	6146[a]	8	1.30
1959	5957[a]	13	2.18
1950–1959	58,231	116	1.99

[a] Preliminary figures.

study the actual incidence of hypertrophic pyloric stenosis met with success. It is a great pleasure for me to extend to these two colleagues and their collaborators my sincere gratitude for their help and assistance.

The period chosen was the 10 years from 1950 to 1959 inclusive. All records of cases treated during this time at the Medical and Surgical Department of the Children's Hospital for hypertrophic pyloric stenosis were studied and the diagnoses affirmed, and the 54,438 records of the infants attending the Welfare Centres studied in order to ascertain if there had been any infant with evident or suspect pyloric stenosis that had been overlooked as not registered in the Hospital. No such instance was found. A questionnaire was sent to the practising pediatricians in Gothenburg asking them for information about cases of pyloric stenosis that had not been admitted to the Children's Hospital. All the pediatricians kindly replied to my demand and in this way an additional number of 3 cases with roentgenologically confirmed diagnosis were found.

Results

In all there had occurred 116 cases of hypertrophic pyloric stenosis during the period mentioned, which gives an incidence of 1.99 per 1000 live births (Table 1). This figure is only about half of that of the previous period and would suggest a decrease in the incidence. The registered number of cases in the different years is seen in Table 1. It varies between 1.04 and 2.55 per 1000 live births. In the 1934–1940 year period there was only one year with an incidence as low (2.5 per 1000), as the

highest incidence during the period 1950–1959 (2.55 per 1000). Chi-square analysis shows the decrease in frequency to be statistically highly significant ($P = 0.0005$). The conclusion is obvious: hypertrophic pyloric stenosis has become less frequent in Gothenburg.

Discussion

The first cause to be excluded when there is a demonstrated decrease of the number of recorded cases of a disease is that there has occurred a registration error. Some cases may have escaped the diagnosis and hence become tabulated as other diseases. This seems to be very unlikely with a disease such as hypertrophic pyloric stenosis, because the symptoms and signs and development are so typical in manifest cases. On the contrary, a diagnosis of hypertrophic pyloric stenosis in a young infant suffering from vomiting when the illness does not exist, seems more probable and should have given an increased number of recorded cases instead of a smaller number.

Can the decreased incidence of recorded cases depend upon the fact that many more manifest cases are treated by private practitioners at home without X-ray diagnosis or observation at the Children's Hospital? A questionnaire sent to the practising pediatricians in Gothenburg has not given any support to this suggestion. As already mentioned, 3 cases of hypertrophic pyloric stenosis had been treated by a private practitioner and in these 3 cases the diagnosis had been confirmed by X-ray examination. Apparently the manner in which suspect and confirmed cases of hypertrophic pyloric stenosis is handled now is roughly the same as in the 1930's. If any case has escaped the attention of the recorder, which, of course, cannot be excluded with certainty, the human factor being always present, the number of cases overlooked cannot be so great that it approaches the annual total number of recorded cases; if such was the case, then there should be about as many overlooked as registered cases in the 1950's, which certainly is unlikely.

Another circumstance that speaks against the lower incidence being due to faulty registration are the notes in the records of the Infant Welfare Centres. The attendance at the Centres was 80 % of all infants born alive in Gothenburg in 1934–1940. In 1950–1959 it was 93.5 %. All infants with symptoms of vomiting and other suspect symptoms and signs during the first 3 months of life have been followed-up. Some of them suffered from hypertrophic pyloric stenosis and were treated at the Children's Hospital, some were sent to the Hospital for X-ray examination or observation without the diagnosis being confirmed, and in the rest the symptoms rapidly subsided or the subsequent course showed that they could not have been due to hypertrophic pyloric stenosis.

Neither does it seem probable that the severity of the disease should suddenly, between 1940 and 1950, have become less marked to a degree that about half of the cases would not produce any clinical symptoms and signs at all. No less than 31 of the 116 cases of hypertrophic pyloric stenosis were considered so severe that they were transferred to the Surgical Department for operation, while during the previous

period not one single case was operated upon. Of course, this may be due to a different approach to the treatment of the disease during these two periods. The records of the operated cases showed that they were severe and that medical treatment had not met with any success. In the hospital records one finds the same distribution of mild, moderately severe and severe cases as in the 1930's. Although it is not possible to exclude that more cases of hypertrophic pyloric stenosis have run an altogether symptomless course than in 1934–1940, it seems very improbable that such a change in the appearance of the disease could be the explanation of the decreased incidence of recorded cases in 1950–1959.

The occurrence of multiple cases of hypertrophic pyloric stenosis among siblings and in the parents and children shows that there is a genetic cause of the disease. It is also well known that boys are affected about three times as frequently as girls, and if, theoretically speaking, the relation between girls and boys was changed by an enormous supernumerary birth of girls or an enormous increase of perinatal deaths among boys, the total number of cases of hypertrophic pyloric stenosis would decline. There has not occurred such an hypothetical abnormal change in the relative number of girls and boys born alive, and the perinatal mortality has decreased to such an extent that, even if all the deaths had occurred in boys, it could not have produced such a heavily lowered incidence of hypertrophic pyloric stenosis as demonstrated. The total infantile mortality rate was only about 2 %. Even if the total number lost by death in the neonatal period, estimated at about 100, had all been boys, among them at most only one case of hypertrophic pyloric stenosis would have occurred and thus contributed to a decrease by one case of the incidence of the total recorded cases of the disease during the period of study.

There has not occurred any increased race mixture to a degree that could explain the demonstrated change of the frequency of pyloric stenosis. There is neither any known change in the number of children in the families in 1950–1959 as compared with 1934–1940, nor in addition is there any known relationship between the order of the child in the family and the tendency to develop hypertrophic pyloric stenosis.

Summary

The incidence of hypertrophic pyloric stenosis has decreased significantly in Gothenburg from 4 per 1000 live births during the period 1934–1940 to 1.99 per 1000 live births in 1950–1959. The cause of this change is not known.

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CASE REPORT

Klinefelter's Syndrome and Mongolism in the Same Person

by ORLA LEHMANN and HANS FORSSMAN

Department of Pediatrics, Department of Psychiatry, and Institute of Histology, University of Gothenburg, Gothenburg, Sweden

In January, 1959, Lejeune, Gauthier & Turpin demonstrated a small, supernumerary somatic chromosome in cultures of tissue from nine persons with mongolism. The same year Jacobs & Strong reported that the chromosomal sex constitution in Klinefelter's syndrome was XXY. Hence, a chromosome count of 47 has been found in both these disorders.

In April, 1959, a combination of the two disorders in the same person was described for the first time by Ford, Jones, Miller, Mittwoch, Penrose, Ridler & Shapiro. Study of the karyotype in this case revealed 48 chromosomes. Thus the patient had the extra somatic chromosome characteristic of mongolism at the same time as he had the XXY sex constitution. He was sex-chromatin positive. As far as we can make out, no other case of this kind in which the karyotype was studied has been published.

Stimulated by this report of Ford *et al.*, we made tissue cultures and studied the karyotype in a male patient with mongolism who showed clear evidence of hypogonadism. Since no biopsy of testicular tissue or hormonal titration had been performed, the diagnosis of Klinefelter's syndrome was not established before cytologic studies.

Case Report

H.M., born on November 27, 1921, was the 9th of 10 children, born when his mother was 41. It was soon realized that he was backward, and he has spent most of his life in institutions. We examined him in 1957 and 1960, and his status was about the same both times and as follows. He showed the typical signs of mongolism, including the characteristic face and four-finger furrow. Fig. 1–2 should be enough to establish the diagnosis of mongolism. He was very backward. He could walk, but could barely manage to feed himself and did not talk at all except to say his own first name now and then. He could control his urination and defecation for the most part, but not always. His intellectual development corresponded to an I.Q. of about 15 (Terman–Merrill).

He showed distinct signs of hypogonadism. His testes were descended, soft and the size of a large hazel-nut. He had only scanty pubic hair and only occasional hairs in his armpits. No prostate could be felt on rectal examination. The fat was distributed in the feminine manner with an accumulation over the buttocks and breasts (Fig. 1). He was slightly knock-kneed. His extremities were not particularly long, which was not surprising in view of the mongolism.

The daily urinary output of the total gonadotrophins was approximately 96 mouse units, which is an abnormally high value.

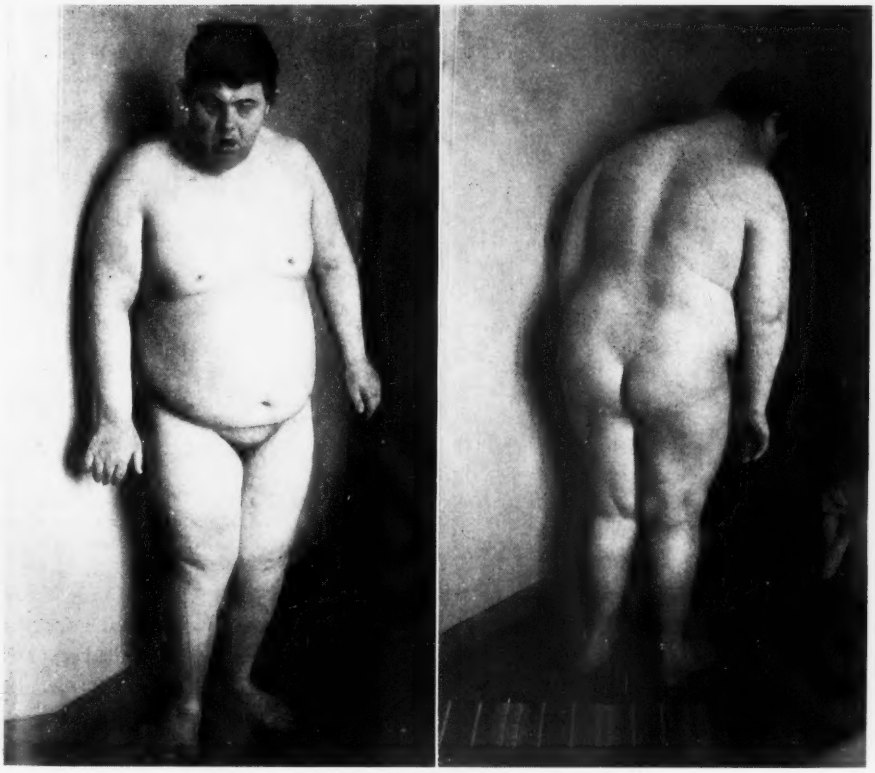

Fig. 1. H. M.

Cytologic Observations

Drumstick analysis of 600 leukocytes in blood smears showed a sex-chromatin-negative pattern. Since the diagnosis was established by the karyotype, we did not feel justified in making a testicular biopsy.

Bone-marrow cells were cultivated according to the technique described by Fraccaro, Kaijser & Lindsten. This revealed that the chromosomes numbered 48. Three mitoses in metaphase showed good separation, allowing both accurate counting and charting. Fig. 3 shows the charting of one of them. As seen, our case also showed an XXY constitution, and a small, single acrocentric chromosome.

Discussion

This is the second time that mongolism and Klinefelter's syndrome have been observed in association. Both cases were studied cytologically, and showed the same karyotype. Mosier, Scott & Cotter made a number of observations of great interest to the question of the connection between Klinefelter's syndrome and mongolism. They described three sex-chromatin-positive men with characteristics of Klinefelter's syndrome. One of the men had a few features suggesting mongolism, another had a half brother who had mon-

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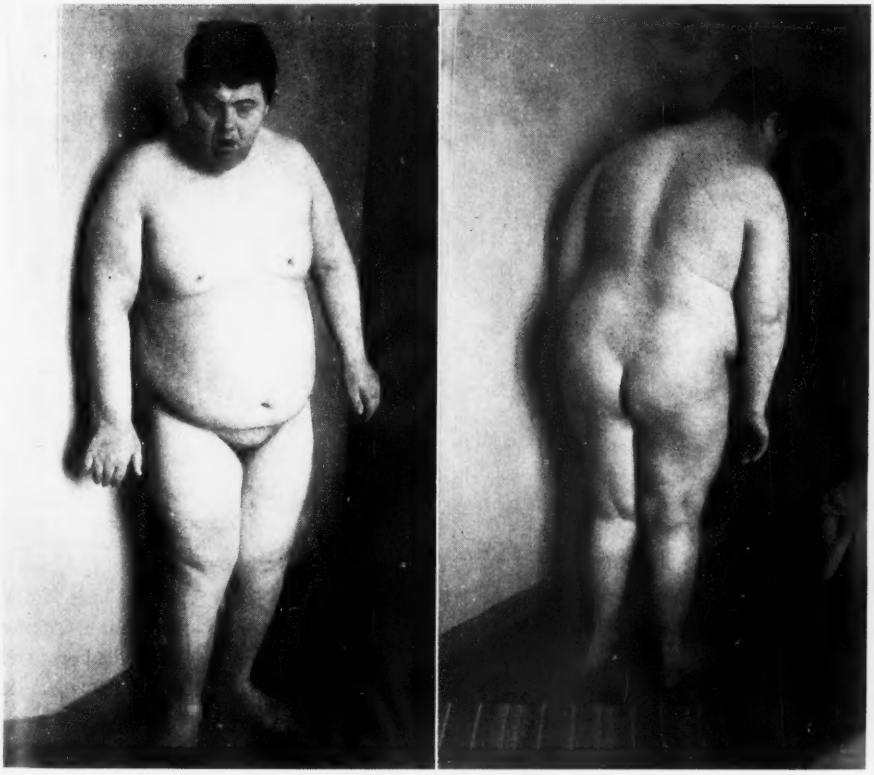

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Fig. 2. H. M.'s hand.

Fig. 3. Idiogram of the diploid chromosome complement. Arrow indicates the trisomic state as found in mongolism. Sex chromosomes in the right lower corner.

golism and the third had twin siblings, one of whom had mongolism.

The fact that the same mother can give birth to one child with Klinefelter's syndrome and another with mongolism, and that one person may be affected by both these disorders, points to an etiologic connection between the two. Lenz, Nowakowski, Prader & Schirren collected the data about parental age from 65 cases of Klinefelter's syndrome and found a maternal age of over 40 in 9, or 13.8 ± 4.3 per cent, of the cases. On the whole, they concluded, advanced maternal age favored the occurrence of Klinefelter's syndrome, but not to such a striking extent as in mongolism.

According to Nelson and Raboch (both quoted from Barr), the nuclei have a female chromatin pattern in about three-fourths of subjects who qualify on clinical grounds for inclusion in the Klinefelter syndrome. The occurrence of cases of sex-chromatin-negative Klinefelter's syndrome and of sex-chromatin-positive Turner's syndrome brings up the question whether one heteropyknotic X chromosome observed in rats may also occur in man, a possibility quite recently suggested by Miles.

Summary

A case of mongolism with the Klinefelter type of hypogonadism is reported. Studies of the karyotype revealed 48 chromosomes, the XXY sex constitution and the trisomic state typical of mongolism. According to the neutrophil test the patient was sex-chromatin negative.

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CASE REPORT

A Family with Marfan's Syndrome Traced through an Affected Newborn

Including Analysis of the Mucoproteins in Serum and the Urinary Excretion of Amino Acids

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Introduction

During the last few years an increasing interest has been taken in Marfan's syndrome especially from a genetical and biochemical point of view. A greater percentage than earlier assumed of more or less pronounced symptoms in families with this disease has been found through careful family studies. McKusick (1) emphasizes that only about 15 per cent of all cases are abortive and that the inheritance in the other cases is due to an autosomal dominant gene with a penetrance which may be very variable.

The triad of symptoms from heart, eyes and skeleton (e.g. dilatation of the ascending aorta with aortic insufficiency or dissecting aneurysms, luxation of the lensis and lengthening of the long bones) is not always present at the same time. Although other authors require all these cardinal symptoms for the diagnosis, it seems to be quite unnecessary in cases with a clear dominant pattern. In many family descriptions, as in the author's, isolated and more or less pronounced symptoms of a connective tissue defect as well as congenital malformations are found in members of Marfan families. Thus they have the disease, but not the whole syndrome.

With the support of histological and experimental studies, McKusick (1) claims that the basic defect may be an elastic fiber abnormality responsible for all symptoms, and that the mutation of only *one* gene may cause the disease. The degree of manifestation may depend on the genetical background of the individual. A family with a pyknic constitution, for instance, may not show any pronounced lengthening of the long bones, which, however, does not protect its members against ocular or cardiac defects. The congenital malformations (heart, skeleton, lungs, eyes, ears, etc.) can only be explained, according to this theory, by the environment in the embryogenesis of generalized pathological connective tissue.

The search for biochemical abnormalities in Marfan's syndrome has been intensified during the last few years in order to

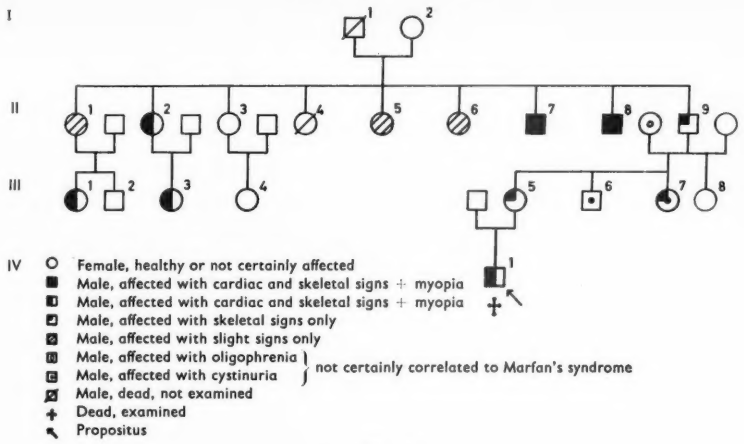
I
II
III
IV
Female, healthy or not certainly affected
Male, affected with cardiac and skeletal signs + myopia
Male, affected with cardiac and skeletal signs + myopia
Male, affected with skeletal signs only
Male, affected with slight signs only
Male, affected with oligophrenia
Male, affected with cystinuria
not certainly correlated to Marfan's syndrome
Male, dead, not examined
Dead, examined
Propositus

Fig. 1.

find a laboratory test for the diagnosis, as well as to make an approach to the pathogenesis. During 1958 reports have been published on increased excretion of hydroxyproline in urine (2), lowered level of serum mucoproteins (3) and altered ratio of chondroitin sulfate to keratosulphate in the costal cartilage (4), findings which may indicate a disturbance of the connective tissue metabolism. No changes in hormonal or electrolyte balance have been found.

The purpose of this report is to gain further experience of the disorder, especially from the pediatric point of view. The above-mentioned changes in blood and urine have also been looked for. It is surprising that after Marfan's investigation so comparatively few pediatric studies have been published on this disease, as it often presents marked symptoms even in the newborn.

Case Reports

The pedigree is shown in the conventional fashion in Fig. 1.

IV_1. The propositus is a male newborn the first child of a 21-year-old woman who has had no abortions earlier and who looked quite normal at first sight. (A club-foot left out of consideration.) The pregnancy was normal, but the delivery, 2 weeks late, was complicated by an abnormal presentation and an intra- and extrauterine asphyxia. Soon after birth the child developed a generalized cyanosis, which increased when he cried. He had very little subcutaneous fat and the skin was strikingly lax. There was a hypermobility in all the joints with a tendency to luxate. A roentgenogram showed a subluxation in the right hip and the shoulders could be forced together under the jaw, as in total absence of the clavicles, indicating a luxation of the palpable clavicles. He also had a pronounced micrognathia and a highly arched palate. The right forefinger was crooked and not flexible. Fingers and toes were rather long.

The cyanosis remained unchanged during the three weeks he was alive. A soft heart murmur developed after a week and the chest roentgenogram showed an enlargement of the heart and increasing densities in the lungs. The child died from heart failure and with signs of respiratory deficiency. An infantile type of coarctatio of the aorta was found at autopsy. The ascending aorta was totally

aplastic and there were only two aortic valves. In the lungs widespread atelectases were found.

The heart failure in combination with signs of connective tissue defect and congenital skeletal deformities indicated the presence of Marfan's syndrome. The diagnosis was further supported by an examination of the mother's family. Heart failures, skeletal deformities and signs of a defect in the elastic tissue showed an inheritance of an autosomal dominant character (cf. Fig. 1). No typical ocular signs were found but myopia was not uncommon.

III_1. The mother of the propositus and sister of III_6 and III_7, 21 years old, seems to have been in rather good health all her life. Her left foot was clubbed at birth. The knee of the same leg has a tendency to dislocation, possibly an effect of the club-foot. At eight years of age she was operated on for an acquired inguinal hernia. On examination it was noticed as a typical Marfan peculiarity that the middle fingers were significantly longer than the other fingers, which had normal length. The palate was highly arched. The systolic heart sound was not clear, but there was no obvious murmur. The elbows were hyperextensible, the skin was rather lax and her breasts hung down to the umbilicus.

In summary, the mother of a child with signs indicating Marfan's disease shows small but typical signs of the disease in skeleton and elastic tissue but has no symptoms from heart or eyes. She is in rather good health and her constitution is not thin and "spidery". The Marfan disorder would probably not have been thought of without knowing her heredity.

III_6. Brother of III_5, 17 years old, has a quite normal body constitution His palate seems to be a little high and the systolic heart sound is not quite clear, but these findings do not seem to be of any pathological significance. The boy is probably not affected by the defective gene.

Surprisingly enough, the urinary excretion of cystine and lysine was much above the upper normal limit.

Fig. 2.

III_7. Sister of III_5 and III_6, 27 years old, has been in good health all her life. At first sight she looked quite normal, but she has small but typical Marfan abnormalities in the skeleton. The sternum is slightly depressed and the chest and head are moderately elongated. She has a highly arched palate and has been operated on for a narrow nose cavity. There are no signs from heart or eyes, but the skin is rather lax and there are, without excessive fat deposition, striae on the hip. She has the Marfan disorder. She has also cystinuria of the same type as her brother (III_6).

III_8. Half-sister of III_{5-7}, was born a week before the propositus, who is the son of her half sister III_5. No symptoms or defects of the Marfan type have been found.

II_9. Father of III_{5-8}, 48 years old, has a rather robust and athletic body constitution

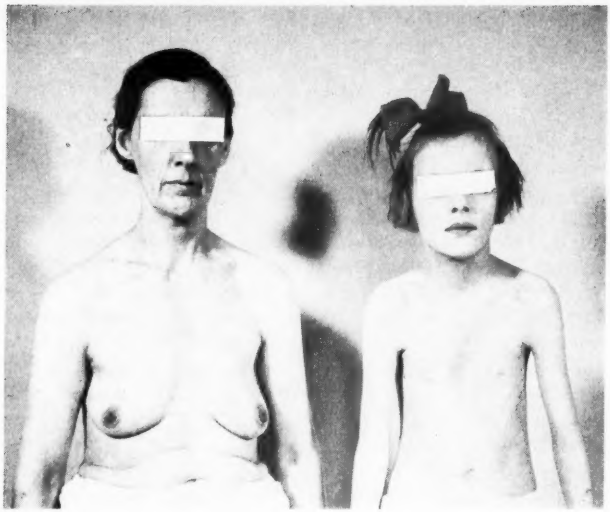

Fig. 3.

and no exterior Marfan signs. He declares he was healthy as a child, but had periods of "rheumatic" pains when he was about 19 and 27 years old. He was at first exempted from military service because of a suspected heart failure and his first joint troubles, but was later allowed to take part in a less strenuous form of military service. He does not seem to have had any typical signs of heart failure or heart pains at that time (no further information is available) and hospital examinations in 1945 and 1959 have not confirmed a heart failure. He has no symptoms from the eyes and a highly arched palate is the only Marfan abnormality that can be found. All joints are normal. In spite of these poor findings he is a carrier of the defective gene, having two affected children out of three in his first marriage. He has many of his father's (I_1) features. His father died from cerebral hemorrhage and is probably not a carrier of the Marfan gene.

Two of his children, III_6 and III_7, have, as mentioned above, a cystinuria that has neither been found in their father's family nor in their mother, who is taken care of in a mental hospital because of oligophrenia.

II_8. Brother of II_9 is 32 years old, but looks at least forty (Fig. 2). Since childhood he has been weak and has done neither physical nor intellectual work of importance. He was not able to go through the elementary school because of mental backwardness and he has not done his military service. He was told that he had a heart failure and a constitution too feeble for it. He had troubles from his joints at the age of fifteen but no further information has been obtained. On account of his bad general state he has received sick relief the last ten years.

On examination the subject was found to have an asthenic body constitution. He was rather thin and the muscles were poorly developed. (Length 181 cm, weight 60 kg.) The chest and extremities were moderately elongated, the fingers long but not "spidery". He had a slight depression of the sternum, the palate was highly arched and the joints were lax.

A systolic murmur was present at the base. Increased jugular pulsations were noticed. Blood pressure was normal. No signs of cardiac incompensation. He had a slight myopia and made an obvious oligophrenic impression.

Like his brother II_7 and sisters II_2, II_5 and II_6, he does not want to leave home for medical examinations. Heart and eyes could, therefore, not be carefully examined with regard to the typical Marfan changes, but the diagnosis seems to be unmistakable, considering the combination of typical skeletal defects, intellectual impairment, suspected heart failure, myopia and the heredity.

II_7: Brother of II_8 and II_9, 46 years old, has always been very thin and feeble and has never done any work. As a child he could not take part in any physical exercise and could not follow primary education at school. He was exempted from military service and told that he had a heart failure. He has received sick relief since 1930. Rheumatic deformities of hands and fingers have developed slowly during the last ten years and he is now not able to take care of himself.

The subject is found to have the most frequent and pronounced signs of the Marfan disorder in his family. The head is long, extremities thin and joints hypermobile. Rheumatic deformities are found in hand and fingers, which are rather long. The big toes are extraordinarily long—a small but typical Marfan defect. There is also a depressed sternum, highly arched palate, pronounced muscular hypotonia and hyperluxible skin. A rough systolic murmur is present at the apex, but no diastolic murmur could be found. Increased jugular and precordial pulsations were noticed. Blood pressure was normal. The diagnosis of Marfan's syndrome was further supported by a myopia and an unmistakable oligophrenia.

II_6: Sister of II_{7-9}, 40 years old, is not married and has no children. She has always been thin and weak. The last few years she has had sharp intermittent pains in the heart region and she very easily gets out of breath. On examination she is found to have a systolic murmur at the base but no other signs of heart disease. There are slightly or moderately pronounced signs of arachnodactylia, dolichostenomelia, pectus excavatum and a rather highly arched palate. The muscles are poorly developed and she is short-sighted. The subject is supposed to have the Marfan disorder, even if the symptoms are less pronounced and some of them may be within normal range.

II_5: Sister of II_6–II_9, 36 years old, has no typical Marfan appearance. As a child she was rather healthy but had a period of pain in the chest at the age of 18 and was then examined by a physician who declared that she had a heart failure. The symptoms disappeared, but the last few years her general condition has declined and she is easily tired and out of breath and she does not exert herself. The only skeletal sign of the Marfan type is a highly arched palate. There is a systolic heart murmur and increased jugular pulsations. She is supposed to have the Marfan disorder, but with slight signs only.

II_4: Sister of II_{5-9}, died at the age of eight months, cause unknown.

II_3: Sister of II_{4-9}, 24 years old, has myopia (4.5 diopters) but is probably not affected by the defective gene.

III_4: Daughter of II_3, 5 years old has had hypertrophic pyloric stenosis in infancy but does not seem to be affected.

II_2: Sister of II_3–II_9 and mother of III_3, 44 years old, presents unmistakable Marfan signs and seems to have a low intelligence. Her attitude towards the examination is negative and she will not give a more detailed description of her childhood. She says she has always been healthy but stammered as a child. During the last few years there have been short periods of pains in the left half of the chest. No trustworthy information of her physical condition can be obtained. Her general state of health is not good. She has a muscular hypotonia and her carriage is bad. The face, fingers and extremities are rather long (Fig. 3, 4). The palate is highly arched and the sternum a little depressed. There is a moderate hyperextensibility in the joints and a tendency in the jaw joints to luxate. A systolic murmur is found at the apex. Because of strabism and myopia she has earlier been examined by an ophthalmologist who could not find any defect in her eyes.

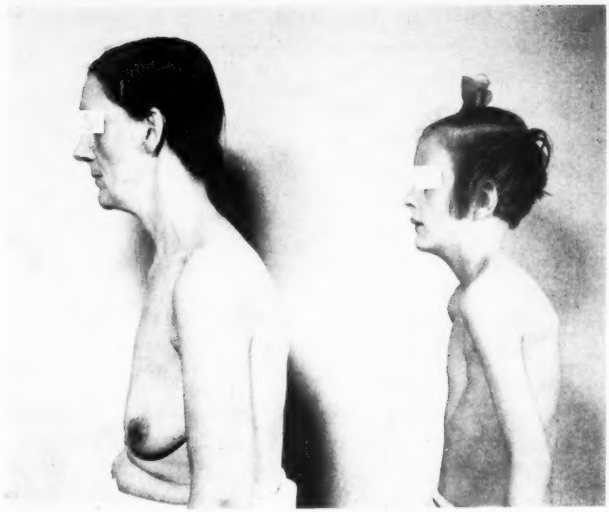

Fig. 4.

The diagnosis of Marfan's syndrome was further confirmed by the examination of the daughter, who is severely affected.

III_3: Daughter of II_2, 11 years old, was born 4 weeks before expected delivery. Birth weight 2250 g. According to her mother no complications after birth. From the maternity hospital she was sent to a children's home because she was very thin and in a bad general state of health. After ten months there she was sent home, her general condition having improved somewhat. She could not walk by herself until she was 2 years old and only when she was 4 did she begin to pronounce separate words. At the age of 8 she started school. She was soon transferred to a class for mentally retarded children in which she has made but little progress. She has been in hospital only for insignificant infections.

On examination the child seems to be seriously retarded. Movements stereotype, answers monosyllabic. She is emaciated and the extremities are very long and thin (Fig. 3, 4). The fingers have the "spidery" appearance typical of Marfan's syndrome. She has a deep funnel chest and a stiff back without lumbar lordosis. The palate is "gothically" arched. There is a pronounced muscular hypotonia and hypermobility of the joints. She has no inguinal hernia, but a broad diastasis is found between the long, straight muscles of the abdomen.

She was examined by an ophthalmologist in 1955 and 1956. He found a divergent strabismus, myopia of the left eye and in the right eye synechiae of the iris, presumably deriving from an earlier iridocyclitis. No other abnormalities were found, but then the typical Marfan abnormalities were not searched for, as Marfan's syndrome had never been thought of. Yet the patient's appearance is quite typical.

II_1. Sister of II_{2-9} and mother of III_1 and III_2, 28 years old, has been in good health until last year, when her general condition declined. She then complained that physical exercise gave her pains in the heart region and that she often felt sudden, sharp pains in the chest, especially at night. The examiner remitted her to the regional hospital for a suspected heart disease. A soft systolic murmur was found, but not other pathological changes. Roentgenograms of the heart,

TABLE 1.

Case	Sex	Age years	Heart failure	Arachno-dactylia	Dolicho-stenomelia	Highly arched palate	Depressed sternum	Other skeleton deformities	Hypermobility of joints	Muscular hypotonia	Myopia	Oligophrenia
I_2	F	69			+	+					+	
II_1	F	28	**		+	+						
II_2	F	44	*	+ +	+ +	+ +	+	+	+ +	+	+	+
II_3	F	24									+ +	
II_5	F	36	**			+						
II_6	F	40	**	+	+	+ +	+ +			+ +	+	
II_7	M	46	**	+	+ +	+ +	+	+ +	+ +	+ + +	+ +	+ +
II_8	M	32	**	+	+ +	+	+		+	+ +	+	+ +
II_9	M	48	*			+ +						
III_1	F	5	***		+	+ +		+	+ +			
III_2	M	7							+			
III_3	F	11	*	+ + +	+ + +	+ +	+ + +	+	+ +	+ +	+	+ + +
III_4	F	5							+			
III_5	F	21	*	+		+ +		+ +	+			
III_6	M	17	*			+						
III_7	F	27			+ +	+ +	+	+				
III_8	F	$^6/_{12}$										
IV_1	M	$^3/_{12}$	***	+		+ +		+ + +	+ + +	+ + +		

Heart failure: * indicates heart murmur without diagnostic significance. ** indicates heart murmur with slight cardiac symptoms. *** indicates heart murmur with severe cardiac symptoms.
+ indicates slightly affected by a particular sign (Not certainly of diagnostic significance). + + indicates moderately affected by a particular sign. + + + indicates severely affected by a particular sign.

lungs and spinal column were normal. ECG and blood pressure were also within normal limits. As the subject is a carrier of the Marfan gene, her symptoms are strongly suspected to come from a heart disease or a dissecting aortic aneurysm. Her constitution is rather asthenic and the palate more than normally arched. She is a gene carrier with slight or uncertain Marfan signs.

III_1. Daughter of II_1 and sister of III_2, 5 years old, was operated on for a persistent ductus arteriosus at the age of 3. A systolic murmur was first heard when she was 6 months old and before the operation she had been easily tired. According to her mother she often stumbles and falls. The joints are hypermobile, especially the ankles. The skin is lax. A moderate micrognathia and a highly arched palate are also noticed on examination. Intelligence and visus are normal. Her appearance is not Marfan-like, but her defects are typical of an affected child who has not arrived at the age when the long bones grow fast.

III_2. Son of II_1 and brother of III_1, 7 years old, has always been healthy. His joints are a little lax but probably within normal limits. He does not seem to be affected.

I_2. Mother of II_{7-9}, 69 years old, says she has always been in good health and has never been to see a doctor. She presents, however, some of the features found in her affected children: asthenic body constitution, a moderate elevation of the palate and a slight myopia. Yet, those signs lying within normal variations, the Marfan diagnosis can today neither be proved, nor dismissed.

I_1. Husband of I_2 and father of II_{1-9}, died 80 years old of a cerebral hemorrhage. He suffered from a hypertonia and was operated on for a gastric ulcer. He was square built and

according to his children his features are easily recognised in his daughter II_3, who is unaffected, and in his son II_9, who is a gene carrier and who has a highly arched palate as the only typical Marfan sign.

A survey of the Marfan symptoms in all members of the family is given in Table 1.

Laboratory Data

Serum mucoproteins

The mucoprotein content in serum of five members of the family have been determined according to the turbidimetric method described by Popper *et al.* (11). Four of these five persons were affected by the Marfan syndrome (II_1, II_2, II_7, III_6). The values obtained varied between 124 and 170 mg %, that is they were all somewhat above normal variations.

Urinary excretion of free amino acids

Free amino acids were determined according to Jagenburg (12). No increased urinary excretion of hydroxyproline was found in any of the eleven cases examined (II_1–II_3, II_5, II_7–II_9, III_4–III_7) and there was no increased excretion of the hydroxyproline containing peptides; the examination was performed as described by Jagenburg (12).

However, III_6 and III_7 presented an obviously increased excretion of lysine and cystine. Therefore, the urinary output of amino acids was collected from their mother, who is taken care of in a hospital for mental disorders because of oligophrenia. The determination gave a normal result.

Comments

In this investigation the subjects have been examined in their homes as most of them refused hospital examination. Several of the affected family members have earlier been examined at hospital, usually because of suspected heart failure. Yet, before the birth of the propositus, the Marfan syndrome had not in any case been suggested. No information as to the type of heart failure they suffer from can be obtained from the hospital records, most of the examinations having been made more than 20 years ago. There is no case of sudden death as often described in Marfan's disorder. This may be due to the affected members living a very quiet life. In spite of the fact that the examinations have not shown any luxation of the lens, dissecting aneurysm or aortic insufficiency, the diagnosis of Marfan's syndrome seems unmistakable, considering the combination of typical skeletal signs, heart disease, myopia and, in some cases, oligophrenia.

It is important to note that individuals affected by Marfan's syndrome need not have a grotesque appearance. The examiner has to search carefully for the typical signs, which may easily be overlooked. The basic tissue defect must always be kept in mind.

General appearance. Some authors (5, 6) have tried to get diagnostic help by measuring body proportions, but the individual and antropological variations are, according to them, considerable and the method does not seem to be useful in cases where the Marfan constitution is not obvious.

Of the family examined, only III_3 has the clinical Marfan constitution. It is interesting to observe that the affected grandfather (II_9) of the propositus seems to have carried the athletic features from his father—who has probably not been the gene carrier—to his affected children III_5 and III_7. In other words, the athletic body constitution of the father of II_9

seems to have suppressed the asthenic Marfan characteristics in III_9, III_5 and III_7.

Skeletal signs. A highly arched palate seems to be the most common skeletal deformity in the whole family, followed by an elongated chest with depression of sternum. The fingers of the affected subjects are usually long, but only III_3 has the well-known spider-like fingers. Clinodactylia and prolongation of the big toe and the middle finger are typical peculiarities also noticed.

The congenital defects often seen in the Marfan disorder were represented of the two cases of micrognathia and one case of club-foot.

Cardiac signs. Systolic murmurs and increased arterial jugular pulsations are common symptoms of the affected individuals. Sharp pains in the chest have occurred in three subjects (II_1, II_5, II_6) and may be signs of slowly dissecting aneurysms. The normal roentgenogram of the thorax of II_1 does not exclude this diagnosis. Aneurysm of the abdominal aorta is not uncommon (7).

The two congenital heart defects are found in this family, e.g. persistent ductus arteriosus and aortic hypoplasia, defects described in other communications (8, 9).

Ocular signs. Myopia is rather usual in the affected members of the family. A more characteristic and less common ocular sign is a sequel of an iridocyclitis found in III_3. This complication is pointed out in a survey of Marfan symptoms by Mc Kusick (1).

Ectopia lentis is not a necessary attribute of the Marfan disorder. A family without typical eye defects was investigated by Wilson (10).

Mental signs. Early retardation, presumably deriving from a primary oligophrenia, was present in 3 cases. Anxiousness, distrust and a long isolation have probably also impaired the intellectual development of these individuals in the later stages of life.

Pediatric aspects. Other Marfan descriptions and the examiners observations show that the main symptoms of the Marfan disorder in infancy are: hypermobile joints, lax skin, herniae or other signs of a defect in the elastic tissue (elastic filaments). Congenital defects of heart, skeleton, teeth etc. are not uncommon. Among congenital heart diseases interatrial defects and coarctatio aortae are the more frequent. In the skeleton primary ossification of the epiphyses of the hands may be present.

The fingers, toes and extremities grow during childhood abnormally long and may give the child as early as school age a thin and tall appearance. The highly arched plate may be present at birth but the funnel or pigeon chest develops generally later, when the ribs grow fast. Thus, some of the typical Marfan symptoms appear during puberty when the individuals affected generally develop long, asthenic features. On the other hand, the joints, according to some subjects, get more stable at the age of 15–20. Experimental studies on animals have shown that the injury to the periosteum leads to an increased lengthening of the growing bone. A possibly decreased elasticity of the periosteum in Marfan's disorder is supposed to have the same effect.

Dislocation of the lenses does not generally start before school age even if it may be present at birth. Iridonesis and turbidity of the lower part of the lens are often

the first signs. As a rule operation will later on be indicated. Many other abnormalities of the eyes are connected with the Marfan disorder (1).

The acquired heart disease is very seldom seen before the age of five. Sudden death owing to heart failure is then observed to increase with age. In some instances there may be congenital heart disease, such as in the propositus.

The Marfan-affected individual is often suceptible to recurrent infections of the throat and lungs.

Biochemical findings. When studying the level of serum mucoproteins in six siblings in a negro family with Marfan's syndrome, Bacchus (3) found a lowered average value during fast, which was statistically significant. This finding suggested changes in the connective tissue metabolism. It has not been possible to confirm these findings either in this study or in six other Marfan patients newly examined by the author. Thus, the alteration of serum mucoproteins seems not to exist in all Marfan patients, and has hitherto only been described in one family.

Sjoerdsma *et al.* (2) found that seven out of ten cases of Marfan's syndrome had a high urinary excretion of hydroxyproline. This amino acid is present in the body almost entirely in collagen. The authors stated that at least some Marfan patients have abnormal excretion of hydroxyproline. This alteration has not been confirmed by the author, but cystinuria in two members of the family studied could possibly be connected with disturbance of the collagen metabolism.

The different biochemical changes in Marfan patients brings up the question whether there may be biochemical variations of this syndrome, due to different enzyme defects as in other types of inborn errors of metabolism. The fact that some Marfan families lack eye and/or heart symptoms support this theory. There may also be variations in the biochemical findings during different ages and with the severity of the symptoms.

Summary

A family with Marfan's disorder involving twelve individuals out of twenty members of four generations is described. The family presents a rich variety of typical skeletal deformities as well as heart disease. Some of the defects are congenital and reported in other publications as connected with the Marfan disease. Eye symptoms are infrequent, myopia is the most common finding.

The propositus was a newborn. Before his birth no member of the family had been diagnosed as affected by the Marfan disease. The search for changes of the mucoprotein content in serum and the excretion of hydroxyproline in urine was negative. An unexpected cystinuria was, however, found in two subjects, one of which is undoubtedly affected by the Marfan disorder.

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Vaccination of Families Against Poliomyelitis by Feeding and by Contact Spread of Living Attenuated Virus

Including Studies of Virus Properties After Human Intestinal Passage

by STANLEY A. PLOTKIN, HILARY KOPROWSKI, SUSANNE N. RICHARDSON and JOSEPH STOKES, JR.

The living attenuated polioviruses are capable of spreading from vaccinated individuals to unvaccinated contacts. This contagiousness is important both with regard to the safety and efficacy of the live-virus poliomyelitis vaccine. The study of viral properties after one or more human-to-human passages can provide data on the stability of the characteristics of the attenuated strains, whereas the immunization of many individuals by vaccine administration to one is of considerable public health significance. Furthermore, through the use of attenuated viruses, the natural history of poliomyelitis infection may be studied.

The first report of transmission of attenuated poliovirus was by Koprowski *et al.* (16) who studied retarded children under specially created conditions of contact. Spread occurred in an environment of high fecal contamination, whereas no spread was observed after vaccination of normal infants in a nursery (17, 26). Moreover, using other strains, Horstmann *et al.* (10, 11) found rapid and extensive spread of virus among retarded children who were allowed to play together, and Martins da Silva *et al.* (29) observed transmission of virus within families.

A family study of vaccination with attenuated polioviruses was organized early in 1958 with three objectives in view:

1) To study the effectiveness of live-virus vaccination in subjects of different ages not living in an institution;

2) To study the transmission of attenuated virus in typical families; and

3) To determine if the properties of the attenuated strains would change after intestinal passages from person to person.

Material and Methods

Organization of the Trial.—Eighteen volunteer families living in Moorestown, New Jersey (a suburban community of metro-

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TABLE 1. *The Ages of the Index Children and Siblings in the Study.*

Ages of index children (in months) and siblings (in years)	Number of	
	Index children	Siblings
1	—	2
2	3	6
3	1	8
4	—	4
5	2	—
6	2	5
7	1	3
8	4	2
9	—	1
10–12	3	2
13–15	2	2
Total	18	35

politan Philadelphia), formed the subjects of this trial. These families had volunteered after attending a meeting at which the entire trial, including its theoretical risks, were explained at length. Socially, these families were of above average intelligence and of average or above average income. Their homes were widely dispersed throughout the town, and there was little or no social contact among them.

Participating in the study were 36 adults and 53 children. Each family consisted of two adults and from one to five children, the average being three. The youngest child in each family, referred to as the "index child", had not received killed-virus vaccine and was without serological evidence of previous natural infection. In Table 1 the ages of the 18 index children and their 35 siblings are given. The index children had a median age of 8 months, with a range of 2–15 months, whereas the siblings ranged in age from 1–14 years, with a median of 4 years.

Before the commencement of the study, index children older than 6 months of age and adults who had not received them previously were given two inoculations of commercial killed-virus vaccine (two weeks apart). At least two weeks were then allowed to pass before living virus was used. All siblings had already been given "Salk" vaccine by their family physicians.

Virus Strains and Vaccine Administration.—The strains employed were CHAT (Type 1), P-712 (Type 2), and Wistar-Fox (Type 3) (18, 29). Virus-containing tissue culture fluids were diluted in saline solution to concentrations of 160,000 50 % tissue culture doses (TCD_{50}) per ml for the P-712 and W-Fox strains and 500,000 TCD_{50} per ml in the case of the CHAT strain. The vaccines were kept frozen until use, when an aliquot was unfrozen and dispensed immediately. For administration, a 1 ml dose of vaccine was added to one or more ounces of milk in a glass or feeding bottle. Considerable care was taken that only the vaccinated individual came into contact with the virus being administered. During the second part of the study some of the adults received gelatin capsules containing a frozen glycerine suspension of virus.

Plan of the Study.—The schedules of virus feeding and specimen collection are shown in Table 2. The first feeding was of Type 1 virus, given to the index children in mid-January, 1958. Type 3 and Type 2 viruses were administered to the index children 3 and 6 weeks later, respectively (Feeding Nos. 2 and 3). Nine weeks after the last feeding to the index child, the entire family was fed Type 1 vaccine (Feeding No. 4). Type 3 and 2 vaccines then were administered 4 and 7 weeks later (Feeding Nos. 5 and 6) to 15 families exclusive of the index children, and to 1 family including the index child.

The families were visited by a nurse twice weekly during the 26 weeks of the study. Prior to the nurse's visits all members of the family collected stool specimens in half-pint containers, which they were asked to refrigerate until the time of collection.

Blood was obtained on 4 occasions: venipuncture was used in adults, whereas in children the specimens were restricted to small amounts of capillary blood. The first specimens were drawn before killed-virus vaccination from those index children and adults who were inoculated with it in preparation for this study. All participants in

TABLE 2. *Schedules of Attenuated Poliovirus Feeding and Specimen Collection.*

Feeding No.	Virus type fed.	Week of trial No.	17 Index children	1 Index child	16 Families	2 Families
1	1	0	x	x		
2	3	3	x	x		
3	2	6	x	x		
4	1	15	x	x	x	x
5	3	19		x	x	
6	2	22		x	x	
Stool collections (Twice weekly)		1–26	x	x	x	x
Throat swabs (With stool collections)		1,4,7	x	x		
Blood specimens		0,15,19	x	x	x	x

the study were bled on the day of administering Type 1 to the index child (Feeding No. 1); 15 weeks later, after all three types had been given to the index child; and lastly, after Type 1 had been given to the entire family (Feeding No. 4). This last bleeding had been scheduled originally for the end of July, after all 6 feedings, but the imminent departure of two families and the fear that "wild" poliovirus might be in evidence by that date prompted us to obtain blood instead after Feeding No. 4.

Pharyngeal swabs were taken from the index children at the two visits during the first week after feeding.

Clinical Observations.—On each visit, the nurse inquired as to the health of the family, and recorded all complaints, however inconsequential. On six occasions during the study the families were visited by a physician, providing opportunity for discussion and elaboration of symptoms. Finally, the families were under the regular care of four local physicians who were fully aware of the nature of the study and the possible significance of illness.

Laboratory.—Twenty per cent stool suspensions were made in Hanks' balanced salt solution (BSS) containing 300 units per ml each of penicillin, streptomycin and mycostatin. After adjustment of the pH to 7.5 if necessary, the suspensions were centrifuged at 5000 rpm for one hour at 4°C. The resulting supernates were recentrifuged (also at 4°C) at 12,000 to 14,000 rpm for another hour. One-tenth ml aliquots from each suspension were inoculated into three monkey kidney tissue culture tubes, each containing 1 ml of Eagle's in Earle's maintenance medium. The tubes were incubated at 37°C and were examined microscopically for cellular destruction daily for 7 days after inoculation. Fluid from tissue cultures which showed no cytopathic changes were blind-passed once by inoculating fresh tubes. Cytopathogenic agents were tested against mixtures of sera prepared in rabbits against the Mahoney, MEF_1, and Saukett viruses in order to identify the poliovirus types present.

If a fecal virus were to be tested for neuropathogenicity in monkeys it was passed once (unless otherwise stated) in monkey kidney tissue culture to increase the titer. Rhesus or Java monkeys weighing 1.8 to 3.15 kg were injected with 1.0 ml of undiluted tissue culture fluid; 0.5 ml into each thalamic area. After 21 days of observation for signs of paresis or paralysis, the monkeys were sacrificed and their spinal cords submitted for histological examination.

Pharyngeal swabs were immersed in saline solution immediately after swabbing. On receipt in the laboratory the swabs were pressed against the side of the tube to ex-

TABLE 3. *Antibodies Provided by "Salk" Vaccination in Moorestown Families.*

Group	No. of inoculations	Pre-vacc. natural antibody	Antibody post-vaccination		
			Type 1	Type 2	Type 3
Index children	2	No	5/12[a] (42)[b]	8/12 (67)	6/12 (50)
Siblings	3	N.T.	18/35 (51)	34/35 (97)	19/35 (54)
Adults	2	No	2/5 (40)	5/6 (83)	4/6 (67)
	2	N.T.	8/14 (57)	14/14 (100)	6/14 (43)
	3	N.T.	8/12 (67)	12/12 (100)	8/12 (67)

[a] Numerator = number with antibody; denominator = number in group.
[b] Numbers in parentheses equal percentage.
N.T. = Not tested.

press most of the fluid and the solutions made up to 3 ml by addition of BSS. Penicillin and streptomycin (450 units of each) were added, and the tubes then incubated at 37°C for one hour. After incubation, the specimens were inoculated into monkey kidney tissue culture in the same manner as stool specimens.

To detect the presence of neutralizing antibodies, the sera were diluted 1:4 and tested by the metabolic inhibition technique (22), for their ability to protect HeLa cells against the cytopathic effect of 100 TCD_{50} of the Mahoney, MEF_1, or Saukett viruses.

Definition of "Susceptible".—As many subjects had received killed-virus vaccine prior to the study, it was not possible in every case to decide whether the presence of antibodies was the result of parenteral antigenic stimulation or of past natural infection. In such cases "susceptibility" was determined retrospectively by the isolation of fecal poliovirus, either from a stool obtained more than 3 days after direct feeding of attenuated virus, or from a stool obtained after contact with a vaccinated individual. Thus susceptibility as defined in this study was not necessarily synonymous with seronegativity, although this was often the case.

Results

Response to Killed-Virus Vaccination.—The antibody responses of the members of these families to formolized vaccine are shown in Table 3. Of 12 index children who received two inoculations, in only 5 (42 %) did antibodies develop to all three types of poliovirus. Of the seronegative adults who were bled before and after receiving two inoculations of killed-virus, 3 of 5 remained negative for Type 1. If all of the antibodies found in the sera of the siblings of the index children are assumed to have been the result of Salk vaccination, the serological response to three inoculations in these children was little better than 50 % for Types 1 and 3. Furthermore, it should be noted that on the basis of the data to be presented, many of the siblings were judged to have had antibodies as a result of previous contact with natural poliovirus.

Response of Susceptible Children and Adults to Live-Virus Vaccination.—In

TABLE 4. *Response to Attenuated Virus of Family Members Without Homotypic Antibody Derived from Natural Infection.*

Group	Homotypic antibody		Fecal virus excretion (Exc.) and antibody response (AB)							
			Type 1		Type 2		Type 3		All types	
	Pre-Salk	Post-Salk	Exc.	AB	Exc.	AB	Exc.	AB	Exc.	AB
Index children (Infants)	0	0	13/13[a] (100)[b]	13/13 (100)	10/10 (100)	9/10 (90)	12/12 (100)	12/12 (100)	35/35 (100)	34/35 (97)
	0	+	5/5 (100)	—	8/8 (100)	—	6/6 (100)	—	19/19 (100)	—
		Total	18/18 (100)	13/13 (100)	18/18 (100)	9/10 (90)	18/18 (100)	12/12 (100)	54/54 (100)	34/35 (97)
Siblings	N.T.	0	8/9 (89)	8/9 (89)	0/1 (—)	—	13/13 (100)	—	21/23 (91)	8/9[c] (89)
Adults	0 or N.T.	0	4/9 (44)	5/9 (56)	—	—	6/10 (60)	—	10/19 (52)	5/9[c] (56)
	0	+	1/3 (33)	—	1/6 (16)	—	3/4 (75)	—	5/13 (39)	—
		Total	5/12 (42)	5/9 (56)	1/6 (16)	—	9/14 (64)	—	15/32 (47)	5/9[c] (56)

[a] Numerator = number excreting or responding; denominator = number without homotypic antibody at the beginning of the trial.
[b] Numbers in parentheses equal percentage.
N.T. = Not tested.
[c] Type 1 only.

Table 4 are shown the fecal virus isolations and antibody responses after direct feedings of attenuated virus to the index children, and later in the trial to their parents and siblings.

Intestinal infection by each of the 3 types of poliovirus was established in all 18 index children, including those who possessed antibodies from killed-virus vaccination. An antibody response accompanied virus excretion in 34 of 35 (97 %) of instances where antibodies were absent before feeding: the single failure was a Type 2 feeding. Similar results were obtained by direct feeding (later in the study) of seronegative siblings who had escaped contact infection from the index child: virus excretion was observed in 21 of 23 instances (91 %). Post-vaccination antibody determinations were available from this group only for Type 1 virus: of 9 previously negative siblings, 8 were infected (89 %) and all 8 developed antibodies.

In contrast, only 15 of 32 (47 %) feedings to adults who were susceptible resulted in virus excretion, and homotypic antibodies appeared after only 5 of 9 feedings to Type 1 seronegatives.

Spread of Attenuated Virus to Contacts.— Fecally excreted polioviruses of all three types were detected in the stools of family contacts of the vaccinated index children, presumably as a result of the spread of attenuated virus. As seen in Table 5, homotypic infection occurred in 42 % of instances in which a sibling contact and in 11 % of instances in which an adult contact were susceptible to poliovirus infection (as determined by later direct

TABLE 5. *Spread of Attenuated Virus to Susceptible Family Contacts of Vaccinated Infants.*

	Fecal virus excretion (Exc.) in susceptibles and antibody response (AB)							
	Type 1		Type 2		Type 3		Total	
Contacts	Exc.	AB	Exc.	AB	Exc.	AB	Exc.	AB
Siblings (Total)	12/28[a] (43)[c]	6/7[b] (86)	7/19 (37)	—	14/32 (49)	8/8 (100)	33/79 (42)	14/15 (93)
Siblings (Secondary)[d]	9/17 (53)	6/7 (86)	6/12 (50)	—	9/16 (56)	6/6 (100)	24/45 (56)	
Siblings (Tertiary)[e]	3/11 (27)	—	2/7 (29)	—	5/16 (31)	2/2 (100)	10/34 (29)	
Adults	4/18 (22)	3/4 (75)	1/10 (10)	—	0/19 (—)	—	5/47 (11)	3/4 (75)

[a] Numerator = those excreting virus; denominator = susceptibles.
[b] Numerator = those developing antibody response; denominator = previously seronegative excretors.
[c] The numbers in parentheses equal percentage.
[d] First contact infections in families with susceptible siblings.
[e] All contact infections subsequent to first in family.

feeding). The percentages of susceptible siblings who were infected were quite similar for each virus type: 43 %, 37 %, and 44 % for Types 1, 2, and 3 respectively.

In 24 of 45 (53 %) instances where there was at least one susceptible sibling in the family, secondary homotypic contact infection was observed. An attempt was made to obtain a crude rate of tertiary spread (e.g., index child to sibling to adult) by computation of the infection rate *after* the establishment of the first contact infection. Even assuming that all families in which two or more contact infections occurred were examples of tertiary spread, the rate of tertiary infection was only 29 % of susceptible siblings.

Transmission of virus by contact was more variable in adults: four adults, or 22 % of susceptibles, were infected by Type 1; one adult (10 %) was infected by Type 2, but no adult was infected by Type 3 attenuated virus.

Antibody responses to poliovirus infections among contacts are also given in Table 5. Of 15 siblings who lacked them prior to infection, 14 (93 %) developed homotypic antibodies. Three of 4 previously seronegative adults were converted to positive by contact infection.

The most rapid transmission apparently occurred in 2 siblings from whose stools Type 2 virus was isolated only 2 days after feeding of the index child. The latest contact infection took place in a sibling who first developed Type 3 virus excretion 53 days after the vaccination of the index child. The latter excreted detectable Type 3 virus sporadically for 63 days after feeding.

Approximately two-thirds of contact infections were established within 2 weeks of feeding of the index child, as shown in

TABLE 6. *First Appearance of Virus in Feces of Contacts of Index Children.*

No. of weeks post-vacc. of index child	Number of contact infections detected by poliovirus type					
	1	2	3	All	%[a]	Rate[b]
1	2	4	2	8	21	6.4
2	11	1	5	17	66	14.4
3	2	2	3	7	84	6.9
4	—	—	3	3	92	3.2
5–15	1	1	1	3	100	0.3
Total	16	8	14	38		

[a] Cumulative percentage of all infections.
[b] Weekly incidence of contact infection per 100 susceptibles. The number of susceptibles at the beginning of the first week was 126 (Table 5).

Table 6. The infection rate was as high as 14.4 per 100 susceptibles during the second week post-vaccination, but averaged only 0.3 from the 5th to the 15th week.

Four examples of intrafamilial spread of virus given to the index child are contained in Tables 7 through 10. The B family (Table 7) exemplifies the more or less simultaneous onset of excretion of Type 1 virus by three siblings. Infection presumably occurred early in the second week after vaccination of the index child. During the 5th week of the trial, 1 of 3 susceptibles in the family were infected by Type 3 virus.

The second example (Table 8) is one in

TABLE 7. *Transmission of Attenuated Types 1 and 3 Viruses in the B Family.*[a]

Person No.	Age (Years)	Susceptibility to polio types			Week of trial No.					
		1	2	3	1	2	3	4	5	6
B–1					V1		V3			
B–1	2/12	x	x	x	1		<3			
B–2	27	x								
B–3	29	x		x						
B–4	6	x				1	<			
B–5	4	x		x		1			<	
B–6	2	x	x	x		1		<		3

[a] In this and Tables 8–10, person No. 1 is the index child, No. 2 is the father, No. 3 is the mother, and other numbers signify siblings;

x indicates susceptible status (homotypic antibodies absent or derived from killed-virus vaccination);

V1 refers to feeding of attenuated virus (Type 1) to the index child at the time indicated by week of the trial;

1, 2, and 3 correspond to first isolations of poliovirus types from biweekly stools; and the horizontal lines show the duration of virus excretion.

A break in the line indicates stools from which virus was not recovered;
< signifies the last isolation of the particular virus; and
—> signifies that virus excretion continued beyond the time period illustrated. However, no new contact infections occurred in these families after this portion of the trial.

TABLE 8. *Transmission of Types 1 and 3 Attenuated Viruses in the Q Family, Showing Circumstantial Evidence for 3 Intestinal Passages of Type 1.*[a]

Person No.	Age (Years)	Susceptibility to polio types			Week of trial No.					
		1	2	3	1	2	3	4	5	6
Q–1					V1		V3			
Q–1	2/12	x	x	x	1		3			
Q–2	27	x	x							1
Q–3	28	x		x		1				
Q–4	3	x	x	x						3
Q–5	1	x		x		1			3	

[a] See footnotes to Table 7.

which the index child (Q-1) infected its mother (Q-3) and a sibling (Q-5) during the second week after vaccination with CHAT. Twenty-one days after the Type 1 feeding, the index child was fed Type 3 vaccine, subsequent to which Type 1 virus was no longer found in his stools. The sibling and the mother continued to excrete Type 1 virus. Thus when the father's (Q-2) stool became positive for Type 1 37 days after poliovirus had been introduced into the family, the most probable source of his infection was either the mother or sibling by tertiary spread. Table 8 also shows that two siblings (Q-4 and Q-5) were infected with Type 3 virus by contact during the 5th week of the trial. It is interesting to note that on two occasions both Types 1 and 3 polioviruses were detected in the feces of Q-5.

In the H family (Table 9) the index child (H-1) excreted Type 2 virus sporadically for 58 days after feeding. Spread of this type of virus to two siblings was observed; one (H-4) at only 2 days after vaccination of the index child and the other (H-6) 29 days later. At the time subject H-6 became virus positive, H-4 had already ceased to excrete detectable virus, but H-1 was still excreting Type 2 sporadically.

TABLE 9. *Early and Late Transmission of Attenuated Type 2 Virus in H Family.*[a]

Person No.	Age (Years)	Susceptibility to polio types			Week of trial No.					
		1	2	3	7	8	9	10	11	12
H–1					V2					
H–1	8/12	x	x	x	2					
H–2	34	x	x	x						
H–3	32									
H–4	7	x	x	x	2					
H–5	6	x		x						
H–6	3	x	x	x					2	

[a] See footnotes to Table 7.

TABLE 10. *Transmission of Type 3 Attenuated Virus in the I Family, Showing Infection of Four Siblings.*[a]

Person No.	Age (Years)	Susceptibility to polio types			Week of trial No.					
		1	2	3	4	5	6	7	8	9
I–1					V3			V2		
I–1	5/12	x	x	x	3					
								2		
I–2	45		x	x						
I–3[b]	41		x	x						
I–4	11		x	x				3		
I–5	10		x	x				3		
I–6	7	x	x	x				3		
I–7	2	x	x	x			3			

[a] See footnotes to Table 7.
[b] Had illness clinically diagnosed as poliomyelitis while pregnant with I–6.

Type 3 contact infection involving four siblings was seen in the I family (Table 10). The youngest sibling (I-7) was the first who excreted detectable virus at 16 days after vaccination, followed by the oldest sibling (I-4) at 20 days after vaccination, and the other 2 siblings (I-5 and I-6) at 23 days after vaccination. Inasmuch as the index child (I-1) excreted Type 3 virus for 27 days after its ingestion, I-4, I-5, and I-6 may have been infected either by contact with him or by tertiary spread from sibling I-7.

Influence of Inactivated-Virus Vaccination on Spread of Attenuated Virus.—In Table 11 data are analyzed with respect to the possible effect of Salk vaccination of the index child on the transmission of virus. The analysis is made by number of families rather than by number of individuals because inactivated-virus vaccination of the index child is an all or none phenomenon for each family; and because this method eliminates the influence of possible tertiary spread from sibling to sibling in large families. The percentage

TABLE 11. *Killed-Virus Vaccination of Index Child and Virus Spread from Index Child to Other Susceptible Family Members.*

Index child Salk-vaccinated	Polio virus type			
	1	2	3	all
Yes	5/11[a]	5/8	6/11	16/30 (53 %)
No	4/6	2/4	3/5	9/15 (60 %)

[a] Numerator = families in which virus spread occurred; denominator = families in which there were susceptibles.

spread produced was similar for Salk vaccinated (53 %) and for non-Salk vaccinated index children (60 %). The small difference was not statistically significant ($x^2 = .001$; $p > .95$).

Influence of Pharyngeal Virus Excretion on Spread.—Virus was detected in the pharynges of 5 index children during the week after ingestion of Type 1 vaccine, and in 1 each after Types 2 and 3 (Table 12). The possibility was considered that spread might be correlated with the presence of virus in the pharynx of the index child. Only for Type 1 virus were there enough pharyngeal positives to at-

TABLE 12. *Pharyngeal Excretion of Virus by Index Children and Intrafamilial Transmission of Virus.*

Presence of pharyngeal virus	Spread within families of poliovirus type			
	1	2	3	all
Yes	4/5[a] (80)[b]	0/1 (—)	1/1 (100)	5/7 (71)
No	5/12 (42)	7/11 (64)	8/15 (53)	20/38 (53)

[a] Numerator = families in which spread occurred; denominator = families in which index child excreted homotypic virus from the pharynx.
[b] Numbers in parentheses = percentages.

tempt comparison: the results show 80 % intrafamilial spread in pharynx-positive and 42 % in pharynx-negative index children.

To determine if those infants who manifested viral multiplication in their throats also had high titers of fecal virus, one stool from each index child, obtained 7–11 days after Type 1 feeding, was tested for virus concentration. As shown in Table 13, virus concentrations above $10^{4.0}$ TCD_{50}/g were observed in the feces of 3 of 5 pharyngeal excretors, and 6 of 12 non-excretors. As also shown in Table 13, index children who excreted more than $10^{4.0}$ TCD_{50}/g of virus on this sampling were sources of intrafamilial transmission of virus in 6 of 9 families, and 43 % of susceptible individuals, whereas those who excreted less than $10^{4.0}$ TCD_{50}/g caused spread in 3 of 8 families and 20 % of susceptibles.

The concentration of virus excreted by the index children in the families of the four adults who developed contact infection with Type 1 virus, was more than $10^{4.0}$ TCD_{50}/g of feces in every case. Two of the four infected adults were not in contact with an index child known to have virus in its pharynx.

Clinical Observations.—Although the small size of this trial did not permit a true test of safety, every effort was made to detect both major and minor reactions to the live virus vaccine. There were no neurological illnesses, and in particular, no signs of meningeal irritation in any of the subjects. The only serious illness in the Moorestown families was ulcerative colitis in a 25-year-old mother, which developed

TABLE 13. *Relationship Between Presence of Virus in the Pharynx and the Concentration of Virus in the Feces of Index Children to the Production of Intrafamilial Spread.*

TCD_{50} virus per g of feces[a]	Index children		Contact infection	
	Pharynx pos.	Pharynx neg.	Families	Individuals
$> 10^{4.0}$	3	6	6/9[b]	12/28[c] (43)[d]
$10^{3.0}$–$10^{3.9}$	1	3	1/4	2/8 (25)
$< 10^{3.0}$	1	3	2/4	2/12 (16)
Total	5	12	9/17	16/48 (33)

[a] Single stool excreted 7–11 days after feeding.
[b] Numerator = families in which spread occurred; denominator = families in which there were susceptibles.
[c] Numerator = individuals infected; denominator = susceptibles.
[d] Numbers in parentheses = percentages.

TABLE 14. *Onset of Minor Illnesses in Index Children (Showing Lack of Relationship to Vaccination).*

Virus type fed	Illnesses beginning during post-vaccination week No.			
	1	2	3	4
1	2	1	3	5
2	1	3	1	0
3	5	4	3	2
Total	8	8	7	7

before she herself received attenuated poliovirus, and in the absence of demonstrable contact infection from her infant. She eventually recovered sufficiently to continue participation in the study.

Minor illnesses ranged from the usual complement of aches and pains to varicella and erythema infectiosum. Since all index children were infected with living poliovirus, their illnesses were tabulated by the week of onset, to determine if an excessive number occurred during the second week post-vaccination. There was no excess of illness in the index children during any week (Table 14). For other family members the data could be analyzed with respect to the presence or absence of fecal virus at the time of illness. In addition, those illnesses which occurred after direct feeding of vaccine, and those which occurred after contact exposure to first passage virus were separately studied. As shown in Table 15, positive excretors had approximately the same rate of illness as non-excretors; the small differences were statistically insignificant.

Duration of Virus Excretion.—Virus excretion was detected after 54 feedings to index children, and 65 feedings to other family members. The absolute duration of virus excretion could not always be determined because many infections were interrupted or obscured by feeding of another type of virus; but as summarized in Table 16, at least 3 weeks geometric mean duration of excretion was observed for all 3 virus types fed to index children.

TABLE 15. *Incidence of Minor Illnesses in Attenuated Virus-Excreting and Non-Virus-Excreting Family Members.*

	Illnesses by poliovirus type			
	1	2	3	Total
After Virus Feeding				
Excretors	4/26[a] (15)[b c]	1/17 (6)	12/38 (32)	17/81 (21)[c]
Non-excretors	3/45 (7)[c]	8/44 (18)	11/25 (44)	22/114 (19)[c]
After Exposure to Contact Infection				
Excretors	4/16 (25)	3/8 (38)	2/14 (14)	9/38 (24)[c]
Non-excretors	11/45 (24)	7/63 (11)	15/57 (26)	33/165 (20)[c]

[a] Numerator = individuals with minor illnesses; denominator = individuals in particular category.
[b] Numbers in parentheses equal percentage.
[c] Chi-square of comparison not significant.

TABLE 16. *Frequency and Duration of Fecal Virus Excretion in Susceptible Subjects.*

Group	Source of virus	Poliovirus infections, by type				Geometric mean duration of excretion in days, by type			
		1	2	3	All	1	2	3	All
Index children	Feeding	18	18	18	54	23[a]	23	21[a]	23[a]
Siblings	Feeding	15	12	17	44	17[a]	15[a]	30[a]	20[a]
	Contact	12	7	14	33	23	20	25	23
Adults	Feeding	7	4	10	21	18[a]	14	18	17[a]
	Contact	4	1	—	5	32	18	—	29

[a] Signifies that the mean includes periods of viral excretion shortened by feeding of another type of attenuated virus.

After virus feeding of siblings, at least 4 weeks mean excretion was observed for Type 3 virus, and at least 2 weeks for Types 1 and 2. The mean duration of attenuated virus excretion after oral administration to adults was approximately 2 to 3 weeks.

The duration of excretion could be determined accurately for all 38 contact infections in siblings and adults. The mean duration for Types 1, 2, and 3 contact infections in siblings was 23 days, and in adults 29 days. Excretion for 1 week was observed after 1 feeding (Type 1) and 2 contact infections (Type 2), all in siblings. The longest continuous virus excretion was 63 days, recorded in a sibling after Type 2 contact infection.

Resistance to Reinfection.—Resistance to intestinal reinfection was demonstra-

TABLE 17. *Frequency and Duration of Excretion of Attenuated Poliovirus After Revaccination of Previously Infected Subjects.*

Group	Source of previous infection	Ratio of reinfection, by type				Geometric mean duration of excretion by virus type, in days						
						Positive excretors only			Including non-excretors			
		1	2	3	all	1	2	3	1	2	3	all
Index children	Feeding	4/18[a] (22)[b]	-/1 (—)	1/1 (100)	5/20 (25)	12	—	3	2	—	3	2
Siblings	Contact	3/12 (25)	-/7 (—)	6/14 (43)	9/33 (27)	20	—	14	2	—	3	2
Adults	Contact	-/4 (—)	-/1 (—)	— (—)	-/5 (—)	—	—	—	—	—	—	—
Adults	Natural infection	1/7 (14)	1/6 (16)	-/6 (—)	2/19 (11)	15	9	—	1	1	—	1

[a] Numerator = those reinfected; denominator = those refed.
[b] Numbers in parentheses are percentages.

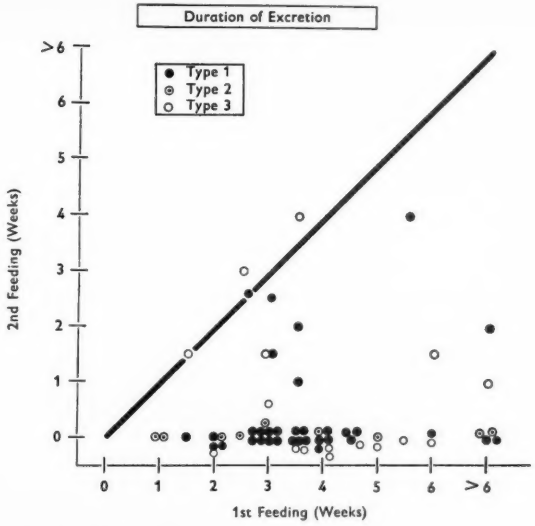

Fig. 1. The duration of fecal virus excretion after first and second feedings of attenuated poliovirus to the same individuals.

ted when attenuated virus was fed for the second time to index children and contact-infected family members, and for the first time to naturally immune adults. Table 17 contains the results of refeeding, which should be compared with the results of first feedings to susceptibles already presented in Table 16. These data are compared graphically in Fig. 1. It will be seen that the duration of fecal virus excretion after second feedings was less than after the first in 54 of 58 cases (93 %), and in 44 cases (76 %) no virus at all was detected after refeeding.

Only 2 of 19 adults who had naturally acquired antibodies excreted virus after feeding of attenuated virus.

Study of Attenuated Viruses After Human-to-Human Intestinal Passages.—The viruses excreted by the index children and their contacts provided material for testing the stability of the vaccine strains with respect to several genetic markers. Three human-to-human passages of Type 1, and two passages of Types 2 and 3 attenuated viruses were tested. These tests have not yet been completed; however, the results obtained thus far are presented here.

The results of intracerebral inoculation of monkeys with undiluted tissue cultures containing from $10^{5.2}$ to $10^{8.7}$ TCD_{50} of vaccine strains or fecal virus are presented in Table 18. The 3 viruses fed showed no intracerebral pathogenicity in these tests. Furthermore, the Type 1 virus (CHAT) failed to produce any paralysis in 14 monkeys, even after 3 human-to-human intestinal passages. The Type 2 virus (P-712) also was clinically harmless for 8 monkeys after two passages. A second human passage of Type 3 virus (W-Fox)

TABLE 18. *Intracerebral Pathogenicity for Monkeys of Attenuated Viruses After Human Intestinal Passages*

Virus inoculum							Pathogenicity		
								Histological lesions	
Type	Source	Human intestinal passage	Isolated days after infection	Tissue culture passage	Log_{10} TCD_{50}	Monkey species	Paralysis	Cervical neurons	Lumbar neurons
1	CHAT[a]	—	—	—	7.5	R	0/5[b]	0,0,0,0,0	0,0,0,0,0
	Q–1	1	7	1	7.0	J	0/4	0,0,0,0	0,0,0,0
	Q–5	2	5	1	5.7	J	0/3	0,0,+	0,0,+
	Q–2	3	12	1	7.5	R	0/3	0,0,0	0,0,0
				2	8.7	R	0/4	0,0,0,0	0,0,0,0
2	P–712[a]	—	—	—	5.2	R	0/5	0,0,0,0,0	0,0,0,0,0
	D–1	1	6	1	7.5	R	0/4	0,0,0,0	0,0,0,0
	D–4	2	5	1	5.5	R	0/4	0,0,0,+	0,0,0,+++
3	W–Fox[a]	—	—	—	7.7	R	0/5	0,0,0,0,0	0,0,0,0,0
	I–1	1	9	1	7.2	R	0/4	0,0,0,0	0,0,0,0
	Q–5	2	22	1	5.2	J	1/4	0,0,+,+[c]	0,0,++,+++[c]
	I–4	2	5	1	5.5	R	0/4	0,0,0,+	0,0,+,+
	Total for Passage Strains						1/34 (3 %)	5/34 (15 %)	6/34 (18 %)

[a] Vaccine strain; results given are for the particular pool used in feeding.
[b] Ratio of monkeys paralyzed to monkeys inoculated.
[c] The monkey with clinical paralysis.
0 = No lesions of poliomyelitis.
\+ = 10–20 % of neurons destroyed.
++ = 20–40 % „ „ „
+++ = 40–60 % „ „ „
++++ = 60–100 % „ „ „
R = Rhesus; J = Java.

paralyzed 1 of 4 monkeys, whereas a first passage virus and another second passage virus failed to produce paralysis in 8 monkeys. Thus only 1 of 34 monkeys developed paralysis after intracerebral inoculation of material representing first to third human passages of these attenuated viruses.

The spinal cord of each of the monkeys was examined histologically: 6 of the 34 had lesions of poliomyelitis; 5 in both the cervical and lumbar areas, and one in the lumbar area only. In none of the positive monkeys was more than 20 % of cervical area neurons affected. With regard to the neurons of the lumbar area, 3 monkeys showed mild histological lesions (< 20 % of neurons affected); 1 monkey showed intermediate damage (20–40 % of neurons); and 2 (including the paralyzed monkey) developed moderately severe neuronal destruction (40–60 %).

The stability of three *in vitro* characteristics of polioviruses was studied for the CHAT Type 1 virus and 9 of its human passage strains. The three characteristics were: comparative growth in tissue culture at 37°C and at 40°C (23), plaque formation in monkey kidney stable line (MS) cells (15), and inhibition of growth in monkey kidney tissue cultures by high dilutions of anti-CHAT serum (31). The results of

TABLE 19. *Genetic and Serologic Markers of CHAT and its Human Passage Strains. (From Koprowski et al. and Gard (8)).*

Strain	Human intestinal passage	Isolated[a] post-infection day No.	Markers[c]		
			Temp.	MS	Neutralization by anti-CHAT serum
CHAT	—	—	Cold	MS	Yes
C–1	1	7	Cold	MS	Yes
D–1	1	8	Cold	MS	Yes
I–1	1	6	Cold	MS	N.T.
L–1	1	7	Cold	MS	Yes
R–1	1	5	Cold	MS	N.T.
Q–1	1	7	Cold	MS	Yes
O–5	2	4	Interm.	MS	Yes
Q–5	2	5	Cold	MS	N.T.
Q–2	3	12	Cold	MS	Yes
Mahoney	—	—	Hot	MS*	No
Virulent (10)[b]	?	10–21	Hot (6) Interm. (1) N.T. (3)	MS* (10)	No (6) N.T. (4)

[a] Isolated in monkey kidney tissue culture and used as first passage.
[b] Ten "wild" strains isolated from paralytic patients during an epidemic.
[c] Explanation of markers:

Cold = Poor growth in tissue cultures incubated at 40°C; 3 or more logs higher titer at 37°C.
Hot = Similar growth at 40°C and 37°C.
Intermediate = Fair growth at 40°C but virus concentration 2 to 3 logs higher at 37°C.
MS = Little or no plaque formation in monkey kidney stable line cells;
MS* = Good plaque formation in both fresh and stable monkey kidney cells.
Neutralization by anti-CHAT serum = signifies inhibition of the strain by the highest dilution of anti-CHAT serum which will inhibit the CHAT strain itself. The tests were performed either in monkey kidney tissue culture tubes or in monolayers under agar.

these tests will be published in detail as part of genetic and serological studies of viruses isolated during a large-scale vaccination program in the Belgian Congo (7, 19), but are summarized in Table 19 for convenient reference. All 9 passage strains which were tested for the temperature and MS characters multiplied better at 37°C than at 40°C ("cold" marker in 7 and "intermediate" marker in 1), and better in primary monkey kidney cells than in the stable line ("MS" character). Serum prepared against CHAT virus completely inhibited plaque formation by 6 passage strains (including 4 first, 1 second, and 1 third passage). In the same tests the CHAT vaccine strain was also "cold", "MS", and inhibited completely by its own antiserum, whereas in contrast, the Mahoney virulent virus and a group of "wild" Type 1 strains isolated from paralyzed patients grew well at 40°C ("hot" marker) and in MS cells ("MS+" marker), and formed plaques under anti-CHAT serum.

At this writing, the W-Fox (Type 3) virus and its first and second human passage strains have been tested only for the temperature character. The original Fox virus grew poorly at 40°C and was therefore a "cold" strain; but in contrast, only one of 4 first passage and none of 8 second passage strains had the "cold" marker.

TABLE 20. *Temperature Markers of Fox And Its Human Passage Strains.*

Strain	Human intestinal passage	Isolated post-infection day No.[a]	Temperature character[a]
Fox	—	—	Cold
I–1	1	7	Cold
L–1	1	9	Hot
Q–1	1	10	Hot
R–1	1	7	Hot
E–4	2	10	Interm.
E–5	2	10	Hot
I–4	2	9	Hot
I–5	2	4	Hot
I–6	2	5	Hot
I–7	2	2	Hot
L–5	2	7	Interm.
Q–5	2	18	Hot
Virulent	?	?	Hot

[a] See footnotes to Table 19.

Of the 3 strains tested for monkey neuropathogenicity, one was "cold" and produced no lesions of poliomyelitis on intracerebral inoculation, whereas 2 were "hot" and also produced lesions in the spinal cords of some of the monkeys.

Surveillance of Poliomyelitis in the Community.—Surveillance of poliomyelitis in New Jersey was maintained with the cooperation of the New Jersey State Board of Health. No case of poliomyelitis or aseptic meningitis has been reported in Moorestown from the inception of this trial to the present writing (November, 1959). Two cases of poliomyelitis occurred during the summer of 1958 in Burlington county, in which Moorestown is located, but both occurred over 20 miles away near the city of Trenton. The nearest cases to Moorestown were reported from the city of Camden, which is 8 miles away, just across the Delaware river from Philadelphia. The Burlington county cases followed by 6 weeks the development of a poliomyelitis focus in Trenton; and the Camden cases occurred after the commencement of the poliomyelitis season in Philadelphia, with which Camden is socially contiguous.

Discussion

The three objectives of this trial were to study vaccine efficacy in normal subjects of different ages; the spread of virus in typical families; and the stability of these attenuated strains after human passage. With regard to efficacy, the strains used were 95 % infectious and antigenic in susceptible infants and children, which is in agreement with previously reported studies of vaccination with the same strains (4, 17, 26). Attenuated virus was as effective after contact infection as after direct feeding, producing antibodies in 93 % of infected children. These results can be contrasted to the relatively poor response of the Moorestown children to the Types 1 and 3 components of the killed virus vaccine.

On the other hand, only approximately 50 % of seronegative adults were infected by live-virus feeding and developed an antibody response. Among the possible reasons for the poor response of the adult are that the gastrointestinal tract of the adult is a less favorable environment for poliovirus multiplication than the gastrointestinal tract of the child; perhaps because gastric acidity in adults destroys much of the virus before it reaches the intestine. Another possibility is that some of the seronegative adults had been infected and immunized in the past by natural virus, but had since lost detectable serum antibodies. Such individuals

might be resistant to infection although apparently seronegative. Relative refractoriness of adults to live-virus infection has been noted also by other workers (2).

The duration of fecal virus excretion was markedly influenced by the poliovirus infection history of the individual. Virus excretion by previously susceptible individuals after a second feeding of attenuated virus was on the average one-tenth as long as after the first infection. Acquisition of intestinal resistance to reinfection by attenuated viruses has been demonstrated previously with these and with other strains (9, 20, 21, 26, 29). Hopefully, the resistance to attenuated virus acquired by vaccinated individuals is associated with similar resistance to challenge by virulent viruses circulating in nature.

Considerable public health significance attaches to the second focus of this study: the ability of living virus to spread and to immunize by contact-infection. A single administration of living virus to the index children immunized, on the average, 1.62 previously susceptible members of these families.

However, our results do not support the concept that "epidemics" of attenuated virus infection will immunize whole populations. Contact infection occurred in roughly 40 % of susceptible siblings for each of the three types of virus, which was a considerably lower rate than the virtually 100 % infection of susceptibles observed in familial episodes of wild poliovirus infection (3, 6, 12) (assuming that the latter were not from a common extrafamilial source). Even after two human-to-human passages, the attenuated viruses maintained their low contagiousness, possibly because the infective dose of attenuated virus remained high.

That only 11 % of susceptible adults were infected by contact perhaps may be explained by the relative resistance of adults to attenuated virus infection, and the better hygiene practiced by them.

Two inoculations of Salk vaccine had no apparent retarding effect on the spread of attenuated poliovirus. In several previous studies, three inoculations of inactivated virus have been observed also to be without such effect (9, 10, 11, 21, 29). There was a trend (statistically insignificant) for greater spread when the index child excreted virus from the pharynx as well as from the intestine. Inasmuch as a considerable degree of spread took place in the absence of detectable pharyngeal excretion, it is obvious that this factor did not exclusively determine the likelihood of spread. Moreover, within the considerable limitations of small numbers it was noted that pharyngeal virus-positives tended to have high concentrations of poliovirus in the stool, which were also associated with a greater degree of spread. Thus we were not able to evaluate separately the importance of pharyngeal and fecal virus excretion in transmission.

The study of live virus vaccination by Gelfand, Potash, LeBlanc & Fox (9) is so cognate to our own that a detailed comparison seems warranted. This group used the LSc (Type 1), P-712 (Type 2), and Leon (Type 3) strains of Sabin (29) in a group of families whose poliomyelitis immunity had been well studied since 1953. Their principal findings were as follows: after virus feedings of $10^{5.1}$ to $10^{5.3}$ TCD_{50} of attenuated virus, 100 % of susceptible children age 2–8 years excre-

ted virus in their stools, and 7 % were also pharynx-positive. With a hundredfold larger dose, fecal virus excretion was maintained, and 74 % had detectable virus in their throat. In comparison, 98 % of susceptible infants and children in the present study excreted virus in the stool. After a feeding of $10^{5.7}$ TCD_{50}, 28 % of infants excreted Type 1 virus in the pharynx in contrast to only 6 % after $10^{5.0}$ TCD_{50} of Types 2 and 3 vaccine. Thus, the rates of intestinal infection in the two studies were quite comparable, and there was some evidence that the incidence of pharyngeal infection varied directly with the virus dose administered.

A striking difference in virus spread (9 % versus 53 %) was observed by Gelfand *et al.* (19) between families belonging to "upper" and "lower" economic groups. In our families, who would all fall into the "upper economic" class defined by Gelfand *et al.*, susceptible children were infected by contact in 42 % of instances. Using the same Type 2 strain, no spread of virus was observed by Gelfand *et al.* among children of "upper economic" New Orleans families, whereas we found contact infection in 37 % of "upper economic" Moorestown children. After taking into consideration possible differences in laboratory technique, two possible explanations for the disparity in results suggest themselves; that the Moorestown families have the characteristics of "lower economic" New Orleans families, or that there was some difference in the "spreading ability" of the two groups of index persons. The first hypothesis can be refuted by our direct observations of the Moorestown families. With regard to the second possibility, it is notable that our index subjects were 2 to 15-month-old infants, whereas children above 2 years of age and adults served this function in the New Orleans study; and that Gard *et al.* (8) observed intrafamilial spread of the CHAT strain by infants under 20 months of age but not by older children. Thus, age of the virus donor may be one of the more significant factors determining the likelihood of transmission.

The third objective of the trial was to study the properties of attenuated viruses after natural transmission from man to man. The relative inability to grow at 40°C or in MS cells (temperature and MS markers) has been found to be generally associated with low pathogenicity for primates on intraneural inoculation (15, 23). No change was discerned in the temperature or MS characteristics of 9 first to third human intestinal passages of the CHAT Type 1 strain. Furthermore, CHAT remained antigenically distinguishable from a group of virulent strains even after 3 human-to-human passages.

Tests of intracerebral pathogenicity for monkeys gave no evidence of a loss of attenuation of the CHAT virus after 3 human intestinal passages. Przesmycki *et al.* (28) also tested 11 strains of fecal virus isolated after one and two human passages of CHAT and found no paralysis after intracerebral inoculation in monkeys: spinal cord lesions occurred only in those injected with a culture of a stool which contained an unidentified cytopathogenic agent as well as poliovirus.

Two human-to-human passages of the P-712 Type 2 strain also failed to show marked changes in its neuropathogenic properties for monkeys.

The results obtained with the W-Fox

Type 3 strain are more difficult to interpret, and larger numbers of monkeys would be desirable. No lesions of poliomyelitis were caused by the vaccine or the first passage strain tested. The 2 second passage strains each produced histologic lesions in 2 of 4 monkeys, but clinical paralysis developed in only one. Polioviruses which produce paralysis in 1 of 4 monkeys after inoculation of 100,000 TCD_{50} have been categorized by others as relatively attenuated strains (11, 13, 14). On the other hand, the temperature marker changed from "cold" to "hot" in the first passage strains, indicating some degree of genetic instability. We conclude that the results reflect no gross change in neuropathogenicity, but that the W-Fox strain is not stable after passage through the human intestinal tract, with respect to certain genetic characteristics, and differs in this respect from the CHAT strain. Further interpretation of the characteristics of the attenuated viruses after passage in these families awaits completion of our tests.

Recent large-scale trials of live poliovirus vaccination (1, 27, 30) have largely allayed fears concerning safety for the vaccinees, but some are concerned with the hypothetical occurrence of backmutation of excreted virus to human neurovirulence and the possible effect of such virus on unvaccinated contacts. For this reason, the stability of the characters of these strains (including attenuation for monkeys) after multiple passage assumes extreme importance. Published reports (5, 24, 25) indicate that a certain proportion of subjects fed other attenuated strains (LSc, Leon KP-34, SM) excrete virus possessing altered characters, including high neuropathogenicity for monkeys. For example, Wesslén & Ekelund (32) recently reported a study in a home for mentally retarded children, where the LSc strain (29) spread from vaccinees to 92 % of susceptible contacts. Tissue culture passages of stools from 2 vaccinees and 8 infected contacts were tested for intracerebral pathogenicity in monkeys. Pathogenicity was demonstrated for strains isolated from 5 subjects. Of 20 monkeys inoculated intrathalamically with these first and second passage strains, 9 (45 %) were paralyzed, whereas the original virus fed had caused no paralysis.

Further research will reveal whether certain strains, such as CHAT, have the specific characteristic of stability through human passage. In any event, it is evident that stability of excreted virus must be evaluated as thoroughly as are other properties of the attenuated strains used for vaccination.

Summary

1. The CHAT (Type 1), P-712 (Type 2), and Wistar-Fox (Type 3) strains of living attenuated poliovirus were administered to 18 infants who were members of typical families living in a suburb of Philadelphia. Asymptomatic intestinal infection with the three poliovirus types occurred in all 18 infants and antibodies developed in all but one infant, who acquired antibodies only to Type 1 and Type 3 viruses.

2. Viruses of all 3 types were excreted by the infants and spread to 42 % of susceptible siblings and 11 % of susceptible adults during the 15 weeks after vaccination. Two killed-virus inoculations given to some of the infants failed to in-

fluence the degree of intrafamilial transmission. The relative importance of pharyngeal and fecal virus in spread could not be determined from the present data.

3. On direct virus feeding, the response of seronegative siblings (ages 1 to 14 years) was similar to that of the infants (91 % infected), whereas seronegative adults were infected by only about 50 % of the feedings. Virus was detected in the stools of susceptibles for an average of 3 to 4 weeks after vaccination. In contrast, previously live-virus vaccinated or naturally infected individuals manifested a high degree of resistance to intestinal reinfection: their average period of excretion was only 2 days.

4. Preliminary tests of fecally excreted virus representing first to third human passage strains were performed by intracerebral inoculation of monkeys. The strains tested failed to show the development of a significant degree of neuropathogenicity. Genetic and antigenic markers of the Type 1 strain also remained stable through 3 human passages, whereas the Type 3 virus became altered in at least one marker.

5. The trial was not designed to test safety, but it was noted that no neurological illnesses occurred, and that minor illnesses were unrelated to the carriage of virus. Surveillance of neurological illness in the area revealed no discernible harmful effect of the trial on the community.

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Treatment of "Hypsarrhythmia" with ACTH

by W. TROJABORG and P. PLUM

Although so-called infantile spasms have been recognized for more than 100 years (West, 1841 (16)) and although this is a frequent form of epilepsy in childhood, only limited interest was shown in this clinical picture until Hess & Neuhaus (10) and Gibbs & Gibbs (7) in the same year, 1952, demonstrated that this type of seizure was associated with a characteristic electroencephalographic picture which Gibbs & Gibbs termed hypsarrhythmia (hypsi = high). The majority of authors state that the condition is for the most part refractory to all the anti-epiliptic drugs usually employed and that the prognosis is extremely poor, viz. 90 per cent of the children become severely mentally defective and 10 per cent die within the first years of life.

In 1958 Sorel & Dusaucy-Bauloye (14) published a report concerning the favourable effect of treatment with ACTH and this was later confirmed by other authors (4, 5, 9, 12, 13, 15). These communications awakened new interest in this disease.

The Authors' Material

The material consists of 30 children observed from 1953 up to and including September 1959. Over half of these cases were observed during the past eighteen months. The group showed a considerable preponderance of boys, viz. 22 boys as compared with eight girls. The age distribution is shown in Fig. 1.

Description of the Seizures

The seizures occur as a series of jerkings with free intervals. The number of jerks per series varies from very few up to about 70. The intervals between jerks may all be equally long but may also vary from a few seconds to several minutes. The jerks most frequently consist of the following components: flexion of the head, symmetrical flexion and adduction of the arms and flexion at the hips of the extended or flexed legs. The contractions occur apparently simultaneously but may be slightly displaced in time in relation to one another, as becomes apparent from slow-motion films (Fig. 2). The seizure is frequently accompanied by screaming or, more rarely, by smiling and occasionally by alterations in colour. In the free intervals, the

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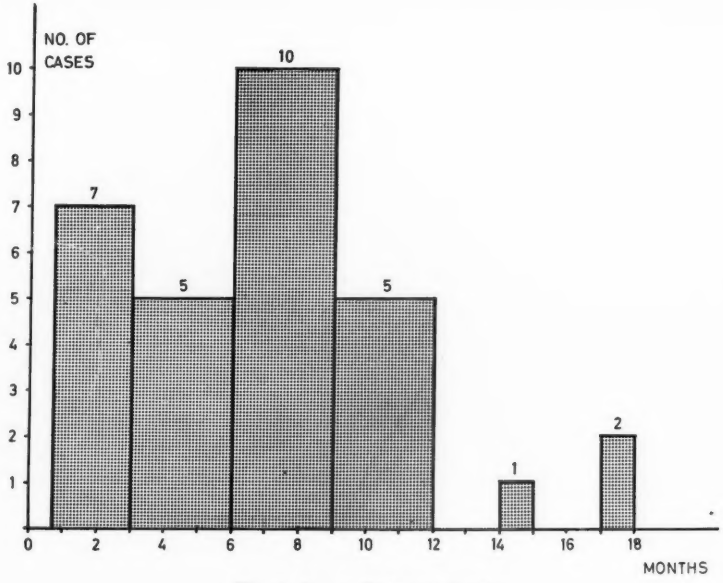

Fig. 1. Age at first examination.

child lies still and apparently relaxed. It is uncertain whether consciousness is lost but the impression is frequently obtained that the child is distressed. The attacks vary in number from very few to approximately ten daily: isolated jerks may, however, also occur.

Experience has revealed that not only the parents but also other observers have difficulty in describing the seizures and it is not unusual that the parents are unaware that fits are involved as the jerks may be interpreted as the expression of pain or ordinary restlessness. The condition may also be misinterpreted by doctors as the attacks may be confused with the absences of petit mal.

A descriptive name for this form of epilepsy is needed. None of the numerous names proposed fully describe the fact

Fig. 2. Section of a film-strip showing an isolated contraction involving the head, arms and legs in a child in whom the attacks consisted, as a rule, of series of 20–30 individual contractions.

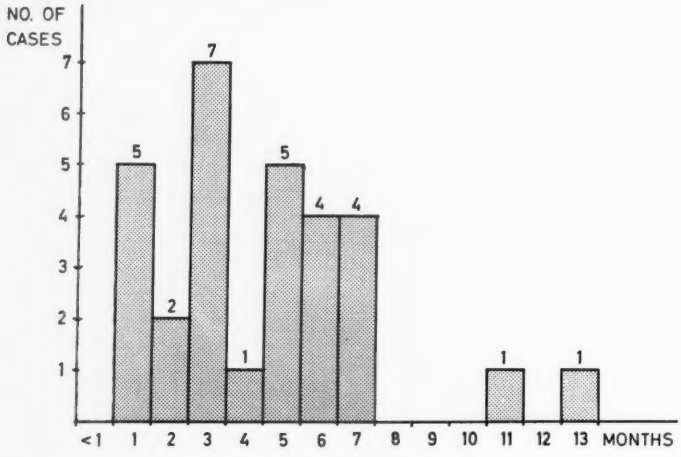

Fig. 3. Age at first attack.

that the symptoms are generalized jerks occuring in series and separated by free intervals. The terms used are epilepsia myoclonica infantilis, infantile spasms, minor motor epilepsy, maladie des spasmes en flexion de la première enfance, encéphalite myoclonique du nourrisson, epilepsie généralisée en flexion and Blitz-, Nick- und Salaamkrämpfe.

Course of the Disease

The attacks begin, as a rule, between the ages of one and 13 months (Fig. 3), and disappear spontaneously at about the age of three to four years or later in some cases. The attacks may affect both apparently healthy children as well as children who have previously presented symptoms of cerebral affection. In the former instance, the parents frequently report that the child has deteriorated both mentally and with respect to motor ability after the onset of the seizures. Of the 30 patients in this material, seven died (23 per cent), all prior to the age of three years (two children died in the first year of life, three in the second and two in the third). Of the remaining 23 children, 19 are definitely mentally defective (83 per cent) and the four remaining children are suspected of being so.

Incidence of the Disease

Whereas Hess & Neuhaus (10) state that infantile spasms, which they term salaam spasms, are relatively frequent in early childhood, the general opinion has been that the condition is rather rare (2, 3, 17). In the Paediatric Clinic of the University Hospital children with infantile spasms constituted 25 per cent of all children from 0–2 years investigated for the first time because of convulsions (Table 1). In this connection, it should be noted that the patient material attending the University Hospital is probably

Fig. 4. The same patient as in Figure 2 at the ages of 11 months and 1 year and 4 months, respectively.

TABLE I. *Number of children suffering from convulsive phenomena investigated for the first time in the University Clinic of Paediatrics, Rigshospital, from 1.3.1958 to 1.3.1959*

	0–23 Months	2–3 11/12 Years	Total
Hypsarrhythmia	15	3	18
Grand Mal	23	11	34
Grand Mal + Petit Mal	1	3	4
Grand Mal + Focal Conv.	2	2	4
Focal Convulsions	6	1	7
Petit Mal	2	1	3
Other Epileptic Attacks	12	3	15
	61	24	85

selected as it consists of the most severe cases and that no account is available of the actual incidence in Denmark.

Etiology

Two of the 30 patients in the material were siblings[1] and in four other cases there was a familial predisposition to epilepsy, in agreement with the findings of Zellweger (17).

In 16 cases the history did not provide evidence of any etiological factor but in four of these 16 cases clinical symptoms of cerebral paresis were encountered. In four others the delivery had been complicated (Caesarean section in two cases and twin delivery in two cases). Of the remaining 14 patients 12 showed neonatal symptoms of encephalopathy and eight suffered from asphyxia at birth. The disease was not preceded in any case by pyrexia which could be interpreted as a sign of encephalitis. Gibbs & Gibbs (8), on the other hand, found encephalitis as an etiological factor in 14 per cent of their cases.

The present material, thus, affords no evidence that infantile spasms differ in etiology from the groups of encephalopathies which develop congenitally or perinatally. Prematurity was present in five cases.

[1] A sister of these patients aged three months has just been admitted on account of suspected epilepsy. The EEG showed a picture typical of hypsarrhythmia. The EEG's of both parents were normal.

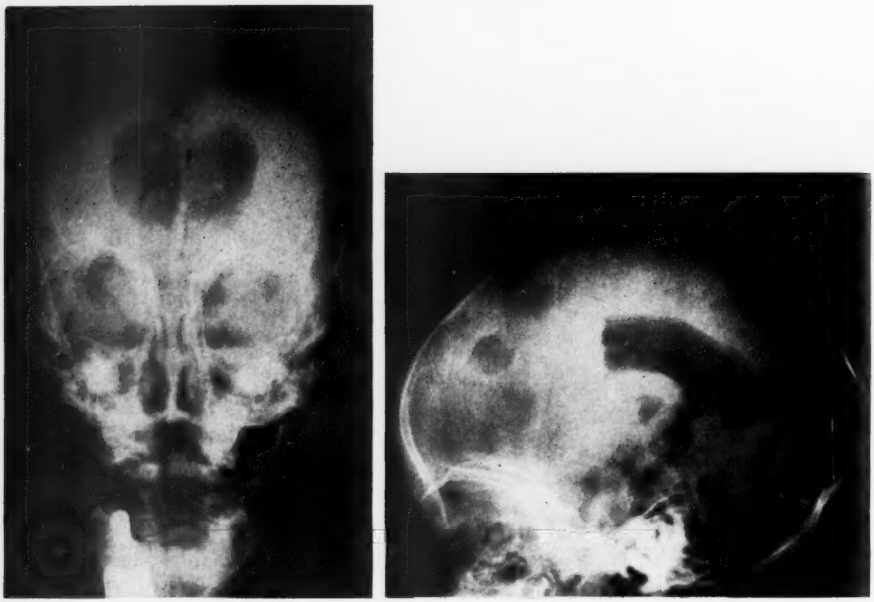

Fig. 5. Pneumoencephalography of the same patient as in Figures 2 and 4 at the age of 1 year and 4 months.

Neurological Investigations

The most striking finding is the oligophrenia mentioned previously. In this material, 26 of the patients were definitely imbecilic and none could be stated with certainty to be mentally normal. No less than 13 out of the 30 children (43 per cent) had cerebral paresis and of these 11 had severe spastic tetraplegia, one spastic hemiplegia, and one spastic paraplegia. Seven of the children had strabismus and of these four were spastics. Microcephaly was found in three patients all of whom were also tetraplegic.

Pneumoencephalography

Air studies demonstrated cortical and ventricular atrophy in all 25 cases in which the examination was performed, in 22 of them, to a considerable extent. In three of the five cases in which the investigation was undertaken on more than one occasion, progressive atrophic changes were found (Fig. 5).

Electroencephalography

The EEG is characterized by slow activity of 1 to 3 per sec of high amplitude (500–600 microvolt) and spikes which occur asynchronously both within the same hemisphere and in both hemispheres. Discharge of spikes may, however, according to Gibbs & Gibbs (7) also be generalized and synchronized. The changes are equally pronounced when awake as during sleep. The picture may be differentiated from

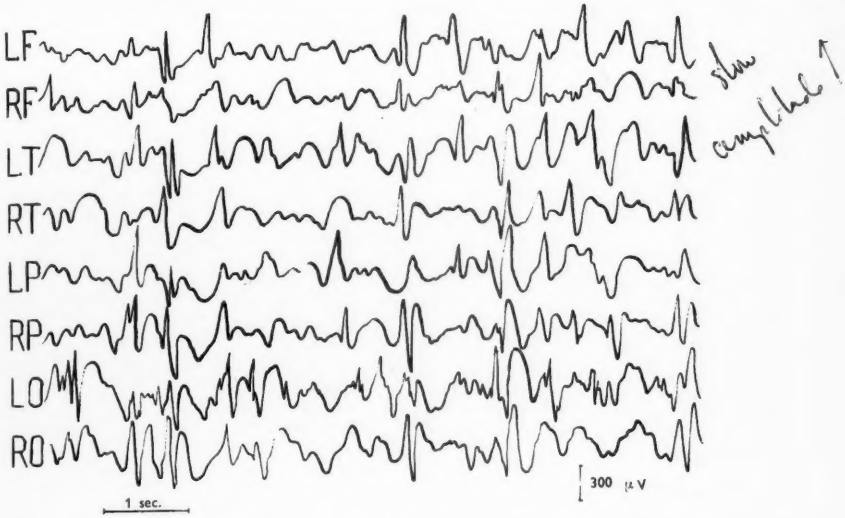

Fig. 6. EEG with changes typical of hypsarrhythmia in a girl aged 14 months recorded one month after the commencement of the attacks. F. = frontal, T. = temporal, P. = parietal, O. = occipital, L. = left, R. = right. Reference = vertex.

petit mal and petit mal variants by the fact that the abnormalities are asynchronous (Fig. 6).

In evaluation of the EEG, the extent to which the spikes occurred syncroneously was investigated and the number expressed as a percentage of the total number of spikes per minute. Out of the 19 cases of typical hypsarrhythmia synchronization of 4–18 per cent was found in 15 cases and from 20–30 per cent in the remaining four cases. Out of these four cases with a more frequent incidence of synchronous spikes, two did not differ with respect to seizures from the 15 cases with a slight tendency to synchronization while in the other two cases, in addition to attacks of infantile spasms, attacks of grand mal were present in one case, and in the other akinetic attacks were observed.

Clinically typical cases of infantile spasms are not always accompanied by the characteristic changes just described as five of 24 cases (20 per cent) of the clinically typical cases showed doubtful EEG findings. Similar observations are found in the literature (10). It is also stated that the EEG may be normal and that this is prognostically a favourable sign (11). The six cases which were considered doubtful on clinical grounds were all atypical electroencephalographically.

Pathological Anatomy

In four of the cases in this material post-mortem examination of the brain was performed and this revealed a fairly uniform picture. Macroscopically, cortical and ventricular atrophy was found and two cases also presented a cyst in the left hemisphere (Fig. 7). Microscopic ex-

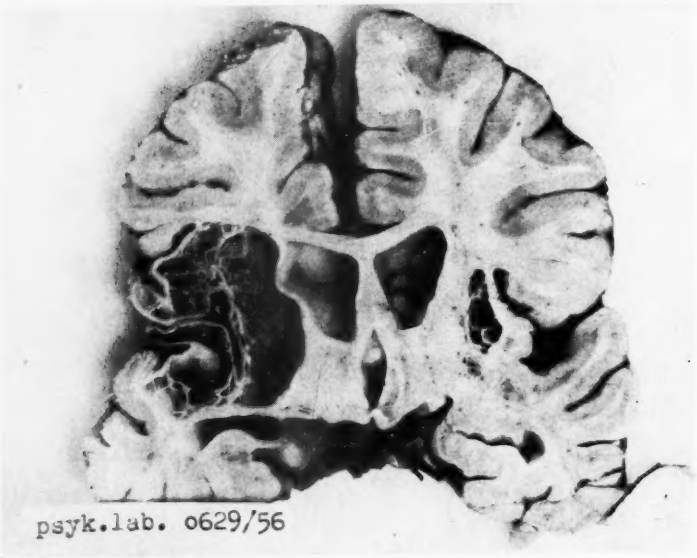

Fig. 7. Cyst in the left temporal lobe in the same patient as in Figure 8.

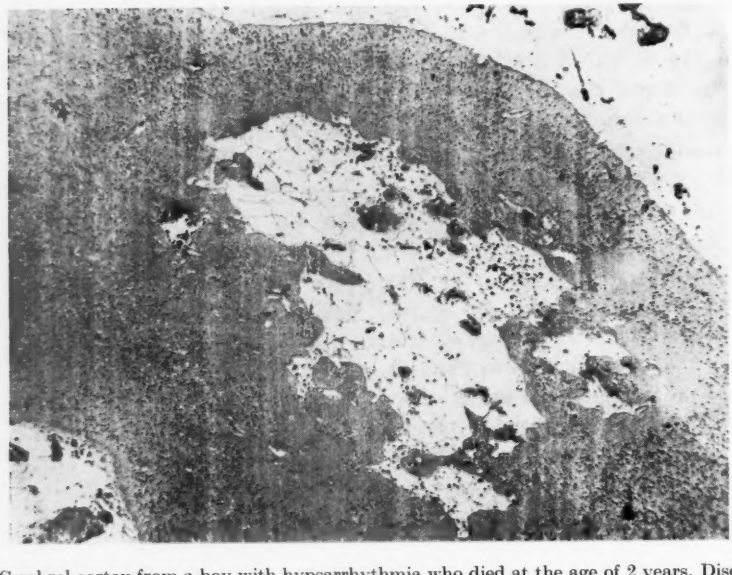

Fig. 8. Cerebral cortex from a boy with hypsarrhythmia who died at the age of 2 years. Disorganization of the cerebral cortex with marked glial proliferation and porencephaly with glial trabeculi and macrophages are present. (HE × 38).

TABLE 2. *Results of ACTH therapy and other treatment.*

Results of ACTH therapy		Results of other drugs	
Clinical effect	7 cases	Clinical effect	2 cases
		No effect	5 cases
Effect on EEG only	3 cases	Effect on EEG only	1 case
		No effect	2 cases
No effect	12 cases	Clinical effect	3 cases
		No effect	9 cases
Total	22 cases		

amination did not reveal any signs of dysplasia but showed diffuse extensive cortical and subcortical degenerative and destructive processes which were partly recent with accumulation of macrophages, and partly older as shown by scars with marked glial proliferation. In two cases, the destructive processes were so pronounced that a state of porencephaly might be said to exist (Fig. 8). Normal conditions were present in the brain stem (Erna Christensen). We have only been able to find reports of three cases of post-mortem examination of the brain in the literature. In one case, atrophy of the cerebellum was found, in the second adhesions of the meninges (Finkelstein 1938 (6)), while in the third case (Bamberger & Matthes (1)) microgyria was present. None of these reports, however, included a description of the microscopic examination of the cortex.

Treatment with ACTH

As a rule acton prolongatum (long acting ACTH) in doses of 5–10 units/day was administered for 3–4 weeks and this course was repeated once or twice if no effect was observed. In some cases, Meticorten or Delcortin were employed instead in a dosage of up to 10 mg daily for up to two months. As a rule phenobarbital (luminal), mysoline (primidone) or diphenylhydantoin (dilantin) were administered simultaneously.

In isolated cases, treatment with ACTH produced a dramatic effect as the attacks ceased in the course of a few days and the EEG became normal. As regards the effect upon the mental state of the children, the present authors and others observed that the children became more alert and easier to contact but in no case was the mental progress so marked that the intelligence could be termed normal. It should be noted that no effect was observed upon the attacks in three cases (all of whom were treated 2–4 months after the commencement of the disease) in whom it was difficult to state with certainty that mental deficiency was present.

As appears from Table 2, 22 children were treated with ACTH and in seven of these (32 per cent) there was a definite clinical effect, i.e. the attacks ceased or diminished considerably in number and severity. Stamps *et al.* (15) describing a series of 60 patients with hypsarrhythmia found a clinical effect in 30 per cent, with ACTH therapy. In three other cases (14 per cent) an effect was observed in the

TABLE 3. *Relation between interval of time from first attack until ACTH therapy and effect of treatment.*

Time between first attack and ACTH	Number of children	Clinical effect	Effect on EEG only	No effect
More than 6 months	7	0	0	7
Six months or less	15	7	3	5
Total	22	7	3	12

TABLE 4. *Relation between the effect of ACTH and the psychomotor development prior to the attacks.*

	Number of children	Clinical effect	Effect on EEG only	No effect
Development normal until first attack	5	3	2	0
Development not normal before first attack	14	2	1	11
Developmental history undertain	3	2	0	1
Total	22	7	3	12

EEG only and in 12 cases (54 per cent) neither clinical nor electroencephalographic effects were observed. It appears further from the table that some effect was obtained with other drugs. In a number of these cases, spontaneous remission cannot be excluded as the improvement occurred late in the course of the disease. The children treated with ACTH were observed from 3–18 months.

It is important to emphasize that ACTH can only be expected to exert an effect if the treatment is instituted early in the course of the disease. This is apparent from Table 3. There appears to be unanimity in the literature on this point. Further, it appears that children who were normal, both mentally and in their motor development, prior to the development of the attacks react better to ACTH than children who were already backward before the first attack (Table 4). This observation has also been made by other authors (4, 14).

TABLE 5. *Effect of ACTH on the EEG.*

	EEG after ACTH therapy				
EEG before treatment	Total	Unchanged	Normalized	Epileptogenic focus	Generalized paroxysmal activity
Typical hypsarrhythmia	12	7	4	0	1
Doubtful hypsarrhythmia	5	2	1	1	1
Not hypsarrhythmia	5	1	3	1	0
Total	22	10	8	2	2

The effect of ACTH upon the EEG is shown in Table 5. The EEG became normal in four out of the 12 children who showed changes typical of hypsarrhythmia. In two of these cases synchronization of spikes of 4–9 per cent was found and in two it was over 20 per cent. These four patients all received ACTH within the first six months after the commencement of the disease. Out of the seven unchanged cases in all of whom synchronization of the spikes was under 18 per cent, four were treated within the first six months while three were treated after the first six months. The material is, however, too small to draw conclusions as regards this finding. In one case, the hypsarrhythmia disappeared and an epileptic focus developed, and in two cases generalized paroxysmal activity developed.

In our cases neitherthe histories nor the pathological findings provide any evidence of inflammatory processes in the etiology of infantile spasms. Nothing is as yet known concerning the mode of action of ACTH in the cases presented; possibly ACTH interferes with an active process of autoimmunizing nature. To achieve favourable effects early institution of treatment is essential.

Summary

An account is given of 30 cases of infantile spasms, a frequent form of epilepsy in infancy, characterized by isolated contractions in the extremities, trunk and head occurring repetitively with brief free intervals and in numerous series daily. Electroencephalographically, a typical picture is found (Hypsarrhythmia, Gibbs) in 80 per cent of the cases.

In our series air encephalography showed atrophy both of cortical and ventricular origin which was progressive in some of the cases. Post-mortem examination of the brain (four cases) revealed pronounced cortical and subcortical degenerative changes with marked glial proliferation.

Twenty two of the patients were treated with ACTH. In seven (32 per cent) definite clinical effect in the form of reduction or cessation of the attacks but no definite effect upon the mental state occurred and in four patients, in addition, normalization of the EEG took place. In three cases (14 per cent) an effect upon the EEG alone was found and 12 patients (54 per cent) showed neither clinical nor electroencephalographic improvement. The possibility that the condition may be due to a cerebral auto-immunizing process is suggested.

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Salmonella Enteritidis Infections in Infancy with Special Reference to a Small Nosocomial Epidemic

by TOV OMLAND and ODD GARDBORG

Salmonella infections in infancy and especially in the newborn often present a very serious clinical picture, in many cases entirely different from the one observed in older individuals. Salmonella types which ordinarily cause only acute gastroenteritis in older children and adults, may in infants cause septicemic infections with a very high mortality. In this connection Salmonella meningitis ought to be emphasized. According to Watson (15) more than 80 per cent of the cases are found in infants, notably in the newborn.

Salmonella meningitis was first described by Ghon in 1908 (4). The infection was caused by Salmonella paratyphi B. Later numerous reports have appeared in the literature and a variety of Salmonella types have been demonstrated as causative agents, the most frequent ones being S. enteritidis, S. paratyphi B, S. havana, S. panama and S. typhi-murium (15).

Bahrenburg & Ecker (2) in 1937, described a case of Salmonella paratyphi B meningitis, a 7-week-old infant, who died 12 hours after admission to hospital. The authors further reviewed 34 cases of Salmonella meningitis from the literature, 31 of which ended fatally. Serological typings had been performed in 19 of the cases with the following results: 12 cases were due to S. enteritidis and 6 cases due to "S. paratyphi". In one case the typing was inconclusive.

Guthrie & Montgomery (6) in 1939, reported 27 cases of Salmonellosis in infants and children caused by S. enteritidis and 1 case caused by S. dublin. The patients were from 36 hours to 2 years of age with one exception, a 13-year-old child. There were 16 deaths, 15 among infants in the neonatal period and one in a 9-month-old child. The clinical picture was characterized by vomiting, diarrhoea, convulsions and coma, and the infection often ended fatally after a few days. The patients who recovered had gastroenteritis without signs of parenteral affection. The authors emphasized the triad; enteritis, cholecystitis and meningitis. At autopsy an enteritis localized to the small intestine was demonstrated in all cases. Meningitis was found in 12, purulent cholecystitis in 4 and moderate signs of cholecystitis in a further 8 of the fatal cases.

The extremely poor prognosis in Salmonella meningitis is also clearly shown in other described epidemics from obstetric and pediatric hospitals (3, 8, 15).

In his excellent review Henderson (7) in 1948, collected 144 cases of Salmonella meningitis from the literature and described a further 3 cases, all infants. The infections were caused by S. newport, S. typhi-murium and S. panama respectively. Levinson *et al.* (11) in 1950, reported 2 cases of meningitis due to S. typhi-murium

and collected a total of 153 cases from the literature. Smith (13) in 1954, described 6 cases of Salmonella meningitis in infancy and reviewed 10 further cases from the recent Anglo-saxon literature. Out of these 16 cases, all treated with antibiotics, 9 recovered, and this represents a great improvement as compared to the nearly 100 per cent mortality in the pre-antibiotic era. The author stressed the need of prolonged treatment because of the risk of relapses. Andrischock (1) in 1954, reported a fatal case of meningitis due to S. paratyphi B in an infant. Agglutinins were found in the patient's blood in spite of their absence in the mother's. The author discussed the theoretical significance of this finding.

Leeder (10) in 1955, described an epidemic caused by S. panama occurring in an obstetrical clinic. The epidemic appeared in 1943–44 and due to unfavourable circumstances (war etc.) it lasted for 9 months before it was finally checked. The causative organism was isolated from a total of 138 persons and among these were 20 newborn infants. Eighteen patients died from Salmonella meningitis and with one exception they all were less than 7 months of age and 13 patients were less than 21 days old. The clinical picture was characterized by diarrhoea, vomiting, fever, convulsions, opisthotonus, rigidity and coma. Four patients had an exanthem similar to the one seen in typhoid fever.

Szanton (14) in 1957, reported an epidemic of Salmonellosis caused by S. oranienburg, involving 46 newborn infants and 34 older individuals. One of the infants died, 7 were acutely ill, 33 had grossly abnormal stools and 5 had asymptomatic infections which were diagnosed by positive stool cultures alone. The epidemic thus had a far less severe character than usual in the newborn, probably because the prognosis depends on the type of Salmonella. The author stressed the fact that the carrier state is prolonged in the infant to a greater degree than in carriers of older age and emphasized the failure of the antibiotic therapy to clear the carrier state.

Watson (15) in 1958 described 4 further cases of Salmonella meningitis and collected a total of 151 cases reported up to that time in the medical literature.

Own Investigations

Epidemiological: In the period from October 31 to December 15 1958 Salmonella enteritidis (1, 9, 12:g, m:—) was isolated from 11 persons in an epidemic originating in the obstetrical department of a Norwegian provincial hospital. Four persons, 1 newborn infant and 3 adults, had no clinical signs or symptoms, the remaining 7 developed more or less severe clinical pictures. One patient, a newborn infant, had meningitis, another patient, a parturient woman, had an uncharacteristic febrile illness, the remaining 5 patients all had gastroenteritis. Two out of the three affected newborn infants died, one from meningitis, the other from gastroenteritis. In the parturient woman mentioned, who had an uncharacteristic febrile illness, agglutinins were demonstrated in the blood (H-titre 1:5160, O-titre 1:160).

Out of the 7 patients 4 (Case 1, 2, 3 & 4) contracted the disease during or in close association with hospitalization in the obstetrical department mentioned, the other 3 (F 1, 2 & 3 ,Table 2) were family contacts.

Among the 4 healthy carriers there were 2 midwives (MI & M2, Table 2) working in the hospital. The first midwife (M1) from whom S. enteritidis was isolated also had agglutinins in her blood (H-titre 1:1280, O-agglutinins not demonstrable). In the second midwife (M2) only traces of agglutinins were found (H-titre 1:40, O-agglutinins not demonstrable). Of the remaining 2 carriers one was a nursemaid

TABLE 1. *Distribution of cases of S. enteritidis infections according to symptoms and age groups. Number of deaths indicated by brackets.*

		Newborn	Older children	Adults	Totals
Manifest disease	Meningitis	1 (1)	0	0	1 (1)
	Gastroenteritis as sole symptom	2 (1)	2 (0)	1 (0)	5 (1)
	Uncharacteristic symptoms	0	0	1 (0)	1 (0)
No manifest disease		1	0	3	4
	Totals	4 (2)	2 (0)	5 (0)	11 (2)

A second probable case (F4, Table 2) has not been included in this table due to lack of bacteriological verification.

TABLE 2. *Chart of nosocomial S. enteritidis epidemic.*

Case 1, 2 & 3, newborns; Case 4, adult; F 1, 2, 3, 4 & 5, family contacts of Case 2; M 1 & 2 midwives; F 6, newborn adopted by midwife M 2.

+ – – – – – – + period with pos.stool samples; + single pos.stool sample; — neg.stool sample; s manifest disease.

	1958 1. Nov.	1. Dec.	1959 1. Jan.	1. Feb.	1. March	1. April
Case 1	s + mors					
Case 2	s + mors					
F 1	s + – – – +					
F 2	s + – – – +					
F 3	s +					
F 4	s —	—				
F 5	+ – – +					
Case 3	s + – – – – – – – +		— — —			
Case 4	s + – – – – – – +				— —	
M 1	+ – – – + — — —					
M 2	+ – – – + — — —					
F 6	+ – + — + + — +					

(F5, Table 2) in one of the infected families, and one (F6, Table 2) was an infant born in the same department and soon afterwards adopted by the midwife M2.

In the only newborn infant who recovered (Case 3, Table 2) antibodies were demonstrated in the blood (H-titre 1:2560, O-agglutinins not demonstrable). This fact is interesting in view of the established opinion that young infants are poor antibody producers (1). However, no blood sample could be obtained from the mother of this infant.

Bacteriological: The technique employed in the bacteriological screening of the stool samples both from the personnel, the contacts, and the patients consisted of culturing on a modification (meat extract instead of placenta extract) of Drigalski agar (9) to which had been added saccharose. The culture was made directly on this medium and

TABLE 3. *Biochemical reactions of S. enteritidis strains.*

Lactose	−	Gelatine liquefaction	−
Mannitol	+	Indole formation	−
Maltose	+	Methyl red reaction	+
Glucose	+	Voges Prosk. reaction	−
Saccharose	−	Citrate utilization	+
Gas formation on carbohydrate media	+	Urease formation	−
		Hydrogen sulph. formation	+

after passage through an enriched medium, Kauffmann's tetrathionate-bile-brilliant-green broth (9). Suspected colonies were examined biochemically and serologically. All strains labelled as S. enteritidis displayed biochemical reactions in conformance with Table 3 above.

The strains further demonstrated a vivid motility and all presented the microscopic picture of Gram-negative short rods with a tendency towards chain formation. The serological typing was performed as slide agglutinations with type factor sera from Behringwerke. All strains showed the antigenic formula 1, 9, 12:g, m:—.

The distribution of positive and negative samples is seen from Table 2. The number of negative samples in the final series is indicated with a—for each such sample. It is seen that some of the patients and the carriers show too few negative results to be considered cleared of the carrier state. This is especially so as regards the family contacts where the line of communication with the persons involved has been indirect, through the local health officer. In the case of the healthy infant carrier (F6) an especially long carrier state is noted and an adequate final negative series is still lacking.

A personnel of 36 has been screened bacteriologically for Salmonella in the stools during the epidemic. It was considered desirable to examine a series of 3 consecutive samples from each member of the staff. On account of rotation etc. only 2 samples were received from part of the personnel and from 4 persons only 1 sample was obtained. The 2 midwives mentioned are exceptions as they were found to be positive and therefore had to be followed for a longer period.

Case histories: As part of the purpose of this article has been to discuss the problem of Salmonella enteritidis infections in the newborn, the case histories of the 3 infants attacked are presented below. They were all born in the above mentioned obstetrical department during the epidemic.

Case I: A full-term female infant (born on October 21 1958) whose birth weight was 3100 g, was admitted to the Childrens' Hospital, University of Oslo, 9 days old. Pregnancy and delivery had been normal. The patient appeared weak after birth and jaundice was noted at 24 hours of age. She was discharged from the obstetrical ward at 6 days of age. A few hours later she developed a fever with elevation of body temperature to 38°C. She was examined by a physician who found a pharyngitis and administered penicillin. During the following days the patient had repeated generalized convulsive seizures with unconsciousness and difficulty in breathing. The abdomen gradually became distended, but the stools remained solid.

On admission the patient was in a critical condition with pronounced signs of dehydration. The rectal temperature was 40,7°C, pulse rate 200 and respiratory rate 100 per

minute. The fontanels were depressed and no rigidity of the neck was present. The abdomen was distended, but peristalsis could be heard. The liver and spleen were not enlarged.

Soon after admission the patient had severe generalized convulsive seizure with unconsciousness, cyanosis and nystagmus. Luminal was administered without effect. Spinal puncture yielded some drops of thick, purulent spinal fluid with traces of blood. During the puncture apnoea occurred with increasing cyanosis. Oxygen treatment and artificial respiration proved futile and death ensued.

Examination of the spinal fluid showed numerous polymorphonuclear leucocytes and a few Gram-negative rods. Bacteriological examination of the spinal fluid yielded growth of S. enteritidis. Further laboratory examinations could not be performed. The Salmonella strain isolated proved sensitive in vitro to sulphonamides, streptomycin, chloramphenicol, the tetracyclines and neomycin, but was resistant to penicillin.

Necropsy findings: The brain surface was edematous and covered with a heavy layer of greenish, fibrinopurulent exudate. Microscopically the leptomeninges were infiltrated with granulocytes. Thrombosed blood vessels were seen in several locations. In the base of the left temporal region a brownish discoloured, partly necrotic area was found, continuing from the surface in the shape of a wedge into the temporal horn of the lateral ventricle. In the other organs no definite pathological findings were seen. The intestines appeared somewhat dilated, but the mucosa everywhere had a normal appearance. Bacteriological examination of pus from the surface of the brain yielded a pure culture of S. enteritidis.

Case 2: A full-term male infant, born on October 28 1958 in the same hospital as the two first cases, was readmitted at 10 days of age to the hospital for gastroenteritis. His birth weight was 3600 g. Pregnancy and delivery had been normal. Jaundice was noted at 36 hours of age.

The patient thrived well the first days of life. On discharge from the obstetrical ward 6 days old his weight had been 3520 g. The first signs of illness appeared in the form of vomiting and diarrhoea with fluid, greenish stools at the age of 7 days. On admission the patient was severely ill with a toxic appearance, dyspnoea, dehydration and pronounced jaundice. Body temperature was normal. The abdomen was not distended and peristalsis could be heard. Liver and spleen were not enlarged. The spinal fluid contained no cells and yielded no growth on bacteriological examination. Radiographs of the chest and abdomen showed nothing of note. Bacteriolgical examination of the stools yielded growth of S. enteritidis.

Treatment consisted of intravenous fluid infusion including blood transfusion, and chloramphenicol per os. The condition, however, worsened and the patient died on the day after admission.

Case 3: A full-term female infant (born on November 4 1958) whose birth weight was 3180 g, was admitted to the Childrens' Hospital, University of Oslo, at 17 days of age for gastroenteritis. This patient is an adopted child and the information is therefore somewhat incomplete. She appeared quite healthy when she arrived in her adoptive parents' home 10 days old. At the age of 16 days she developed diarrhoea with fluid, greenish and very evil-smelling stools. She refused to take food and became increasingly weak. Upon admission the following day the patient was in an extremely poor condition with a toxic appearance and pronounced signs of dehydration. The rectal temperature was 37°C, the pulse rapid and barely palpable, the respiration laboured and deep. The abdomen was slightly distended and peristalsis could be heard. Liver and spleen were not enlarged. There were no signs of meningitis.

The blood picture showed a polymorphonuclear leucocytosis with a total white blood cell count of 29,600. The plasma carbon dioxide content was 9 vol%, the serum chlorides 120 mEq/l, the blood urea 55.6 mg/100 ml. The spinal fluid was clear with negative protein reactions and contained 45/3 cells. A bacteriological examination of

the spinal fuid yielded no growth. S. enteritidis was isolated from the stools. The sensitivity pattern to antibiotics was identical to the one found in the strain isolated from Case 1.

Treatment consisted of fluids intravenously, penicillin and streptomycin parenterally and neomycin per os. A few days later, when the final result of the bacteriological examination was available, streptomycin was exchanged for chloramphenicol per os. The patient received intravenous fluids including blood transfusions for 5 days. During this treatment her condition improved rapidly and after a few days fluid could be given per os. The diarrhoea gradually improved and ceased after one week. The antibiotic treatment could be discontinued after 2 weeks. The patient's condition later on was satisfactory, but it proved difficult to clear her of the carrier state. She was therefore given another intensive 3 weeks' treatment with chloramphenicol and penicillin. Cultures of the stools thereafter became negative for Salmonella.

The patient was discharged after 10 weeks' hospitalization. She was then completely recovered and in an excellent general condition. Antibodies against S. enteritidis could be demonstrated in her serum after 3 weeks of illness (H-titre 1:2560, O-agglutinins not demonstrable).

Comment

From the accumulated facts nothing definite can be said as to how the causative organism had been introduced into the hospital. Theoretically the alternatives of introduction by drinking water, food, a human carrier or a patient with a subclinical infection are equally probable. Gray & Harvey (5) in 1955, described a Salmonella epidemic in a residential nursery where the infection had been conveyed in the milk from a neighbouring farm.

After having discussed the problems with the local public health officer, and especially after having ascertained the absence of known S. enteritidis infections in the community, it seems reasonable to suspect a human carrier. This might be either one of the parturient women admitted to the birth clinic or one of the personnel. In this connection it should be noted that the first midwife, apart from being a carrier, also showed a considerable agglutinin titre in her blood (H-titre 1:1280, O-agglutinins not demonstrable). She had, however, reportedly not been ill for any period associated with this epidemic, and the first stool specimen positive for Salmonella was found nearly 1 month after the epidemic started. The fact that the incidence of lasting carrier states in adults is low in enteric Salmonella infections should be kept in mind.

The mode of spread of the infective agent once introduced into the hospital can also only provide a basis for speculation. It must, however, be evident that the personnel and the practical standard of hygiene within the department must be decisive factors in the secondary spread. As one hardly can eliminate the possibility of introduction of Salmonellae or related organisms into a hospital—in this case an obstetrical department—carried for example with a patient, the consequence must be to aim at a faultless hygienic standard. When an epidemic like the one here reported is already a fact, the most drastic measures must be resorted to, and the complete closure of the institution may be fully justified (10).

In the small nosocomial epidemic de-

scribed here 2 of the 3 affected newborn infants died from their infection and this fact underlines the seriousness of Salmonellosis in this age group. The most characteristic feature of these infections in infancy and particularly in the newborn is the great invasive capacity of the causative organism. Septicemic syndromes are common, often resulting in the development of purulent meningitis with a rapidly fatal course.

Although Salmonella meningitis seems to occur with a low frequency — only about 150 cases reported in the medical literature—the fulminant clinical picture and extremely high mortality emphasize its clinical and epidemiological importance. The disease is far more common in infancy and especially in the newborn than in other age groups. Watson (15) found that out of 138 cases 55 were newborn, a further 31 were under 3 months and an additional 26 under 1 year of age. The prognosis was found to be grave in all age groups and particularly in the neonatal period. In the age group from 0 to 30 days the author found 63 deaths out of a total of 67 cases. This corresponds to a mortality of 94 per cent. In children up to 1 year of age the mortality was 91 per cent and in children over 1 year 70 per cent.

The first patient in the present series is a typical example of a grave Salmonella infection in a newborn infant with purulent meningitis and a fatal course. The rather uncharacteristic clinical picture without signs of enteritis or meningitis demonstrates the diagnostic difficulties. This fact is of particular significance in a disease where early diagnosis and adequate therapy are of decisive importance.

At present it is difficult to judge to what extent the introduction of newer antibiotics and chemotherapeutics has improved the prognosis in Salmonella meningitis. Levinson *et al.* (11) have collected a total of 128 cases from the literature prior to 1938. Of these patients only 8 recovered. From the period after the introduction of sulfonamides and later of the antibiotics the authors have collected 25 cases and of these 12 recovered. The improved prognosis after the introduction of newer chemotherapeutics and antibiotics has also been stressed by Smith (13). Recovery has been described after treatment with sulfonamides, streptomycin, aureomycin and chloramphenicol used alone or in various combinations. The prognosis probably also depends on the type of Salmonella with the highest mortality in infections caused by S. enteritidis, S. typhi-murium and S. havana (15).

The feature that Salmonella infections so often display a severe course in infancy must be ascribed to the generally decreased resistance against infections and poor capacity of antibody production seen in this age group. The intestinal mucosa is supposed to be highly permeable and to possess only slight resistance to microbial invasion. This may explain why newborn infants often develop septicemia in enteric infections with Salmonella types which elicit otherwise only gastroenteritis. On the other hand, it is remarkable that newborn infants may be infected with, and can harbour, pathogenic Salmonella types without any signs or symptoms of disease. In the present series S. enteritidis has been repeatedly isolated from the stools of a newborn infant (F6, Table 2) who has been perfectly healthy the whole time.

Consequently it must be assumed that in this age goup there also exists a considerable individual variation in the degree of resistance, and it is probable that earlier Salmonella infections in the mother may be of significance.

Summary

An epidemic of Salmonella enteritidis infections is reported. The epidemic originated in a maternity clinic during November-December 1958. It involved 11 persons including 4 healthy carriers and 7 patients. Among the latter were 3 newborn infants, 2 of whom died. One of the deaths was caused by a septicemic condition with special localization to the meninges. The other was caused by a severe gastroenteritis with toxemia and dehydration. The infant who recovered also demonstrated a picture of grave gastroenteritis. Treatment consisted of the administration of antibiotics and fluids parenterally.

General comments are made on the clinical features of this type of infection, stressing the significance of the low host resistance in the age group concerned and the diagnostic difficulties. The epidemiological and hygienic problems involved are also discussed with emphasis on the hygienic standard necessary within a hospital and the drastic measures which must be resorted to if infections of this kind penetrate the defense barrier of such institutions.

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Renal aspects of Acid-Base Control in the Newly-born

II. The Organic Acids

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Phosphates have been shown to account for a very small fraction of the titratable acidity of a newborn infant's urine and most of the acidity is contributed by organic acids (4, 12, 12a, 26). Some of the organic acids in the blood and urine of the healthy and of the sick child have been identified, but, until now, no attempt has been made to detect as many as possible of the organic acids which occur physiologically in the blood and urine of newborn infants. For this reason an investigation has now been made, using paper chromatography, of the organic acids in cord blood and in the urine of the foetus and of the child in the first eight days after birth. By identifying the organic acids it has been possible to gain some insight into metabolism and connected phenomena, such as the function of certain enzymatic systems.

Material and Methods

Twenty-three specimens of urine, passed during the first eight days of life, were studied. Collections covering 24 hours were always used. Urine was also collected from seven babies at the time of birth, this urine having been formed *in utero*. It was necessary to pool the foetal urine from all these babies in order to obtain enough material for analysis by the standard procedure. Samples of cord blood from 12 infants were also investigated; some of these infants were those whose urine was also studied.

The urine was collected under toluene, filtered, and kept at −15°C. The amount taken for processing and chromatography was always such that it contained 2 mg of creatinine. This volume was handled as described by Nordmann and co-workers (14, 17). It was first passed through a column of a strongly basic anion exchanger (Dowex 2 X, 200–400 mesh), which had been carefully cleaned beforehand. The anions were dissolved out of the exchanger with 12-N formic acid (30 ml). The eluate was evaporated to dryness in a watch-glass with a hair-dryer at a temperature below 30°C. By regenerating the column carefully it was possible to use it for at least five urines.

The blood was centrifuged immediately and the serum stored at −15°C. After thawing, it was passed through an ultra filtration shell (Membranfiltergesellsch.—Sartorius A. G., Göttingen, Germany), using a suction pressure of about 600 mm Hg applied to the outside. Then the membrane was washed several times with distilled water under the same pressure. The serum ultrafiltrate was treated as described by Nordmann and co-workers

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(19). It was passed through a column of basic anion exchanger in the same way as the urine, which was eluted with 12-N formic acid and the eluate evaporated to dryness. The subsequent treatment of the eluate was the same for both urine and serum.

The desiccated eluate was dissolved in 0.1 ml of water and put on Whatman No. 1 paper (46 cm × 46 cm). Solvent A was a mixture by volume of 80 parts of absolute alcohol, 5 parts of ammonia (21.6 %), 15 parts of distilled water. The time of flow by the descending method ranged from 12 to 13 hours at a room temperature of 19 ± 1°C. The paper was hung up to dry for at least 5 hours. Solvent system B consisted of 50 parts of n-propyl alcohol, 50 of cineole and 20 of conc. formic acid to which distilled water was added drop by drop until the mixture became slightly turbid. This solvent system was left in a separating funnel for at least 24 hours at the temperature at which the paper chromatography was to be made. In this time the milky liquid separated into two layers, the upper of which was the solution used. The lower layer was thrown away. The time of flow ranged from 11 to 12 hours, and subsequently the paper was dried at room temperature for 12 hours.

The paper was developed with an alcoholic solution of brom-cresol green (0.1 %), to which N-sodium hydroxide had been added until the colour changed to blue. With this reagent the organic acids appeared as yellow spots on a blue to dark green background. These yellow spots did not fade in the course of time, but assumed a more vivid hue, while the background could at any time be dyed blue again by ammonia vapour. The acids were identified by their positions on the paper and by the more specific reagents listed by Nordmann and co-workers (13, 14, 16, 18), The R_F values of the individual organic acids are also given by Nordmann, R. *et al.* (18).

Results

Eighteen organic acids were identified in the urines. Two more were tentatively identified and the acid responsible for two of the yellow spots is still unidentified.

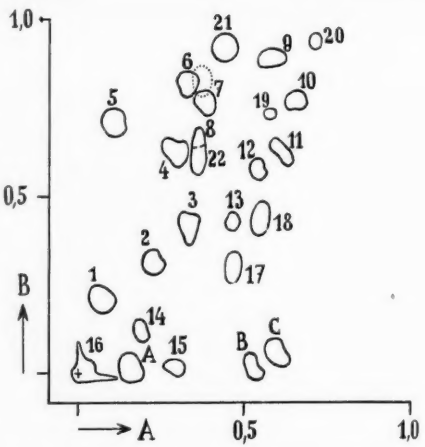


Fig. 1. The position taken up by the identified and unidentified organic acids in the urine formed shortly before and in the first 8 days after birth. 1. Citric acid. 2. Malic acid. 3. α-keto-glutaric acid. 4. Succinic acid. 5. Aconitic acid. 6. Fumaric acid. 7. Adipic acid. 8. Glutaric acid. 9. m-hydroxy-benzoic acid. 10. Hippuric acid. 11. β-hydroxy-butyric acid. 12. Lactic acid. 13. Glycolic acid. 14. Tartaric acid. 15. Uric acid. 16. Oxalic and Phosphoric acid. 17. Pyrrolidone-carboxylic acid. 18. Phenylacetylglutamine. 19. m-hydroxy-hippuric acid. 20. p-hydroxyphenyl-acetic acid and/or β-(m-hydroxyphenyl-) hydracrylic acid. 21. Unidentified spot (no coloration with sulphanilic acid-diazotate.) 22. Unidentified spot.

The spot indicated by the broken circle between 6 and 7 is not visible after spraying with brom-cresol green but gives a pink colour after spraying with n-dimethyl aminobenzaldehyde in acetic anhydride (Gaffney *et al.*, 1954).

A. Sulphuric acid. B. Hydrochloric acid. C. Nitric acid.

Fig. 1 gives the relative positions taken up by the identified and unidentified acids found in foetal and newborn urines. Dent (6) emphasized the importance of showing these relationships, and the R_F values have not been listed for the reasons given by Benson *et al.* (2) and Stark *et al.* (23).

The largest spot was due to citric acid.

It was impossible to separate citric from isocitric acid. The spot corresponding to these acids was elliptical, and their migration in the acid solvent system was impeded by phosphate and oxalic acid.

Malic acid was also represented and near it α-keto-glutaric acid, which showed a slight tailing. To the right of this acid, Fig. 1 indicates a group of three organic acids, glycolic and pyrrolidone carboxylic acids and phenyl acetyl glutamine. The last two acids were identified by the method of Rydon & Smith (21).

Aconitic acid was also excreted. In its vicinity on the paper a group of four acids was identified: succinic, glutaric, adipic, and fumaric acids. The tailing of the glutaric acid is probably caused by the fact that this spot really consists of two almost overlapping spots. The upper spot corresponds to glutaric acid, while the lower section corresponds to a still unidentified acid, which Nordmann frequently found in the blood plasma of anuric patients.

To the right of fumaric acid there was another spot which could not be assigned to any known acid. In solvent system A the R_F value of this acid was 0.44 and in solvent system B it was 0.89. According to Nordmann, who examined our chromatograms, this spot does not correspond to a free phenolic acid, as treatment of the area with sulphanilic acid diazotate produced no coloration.

Immediately above hippuric acid the area taken up by m-hydroxybenzoic acid may be seen and beside it there is a spot which assumed a violet colour with sulphanilic acid diazotate and, according to Nordmann (15), probably was due to p-hydroxyphenyl-acetic acid or to β-(m-hydroxyphenyl)-hydracrylic acid. Beneath hippuric acid there is a spot which, like hippuric acid, assumed an orange colour with n-dimethyl aminobenzaldehyde in acetic anhydride (8) and was probably due to m-hydroxyhippuric acid. The same reagent produced a rose coloration above and between fumaric and adipic acids. This area gave no coloration with brom-cresol green and the substance responsible has not been identified.

The areas taken up by β-hydroxybutyric acid and lactic acid partly overlap. Some of each of these two acids is lost in preparing the urine for investigation.

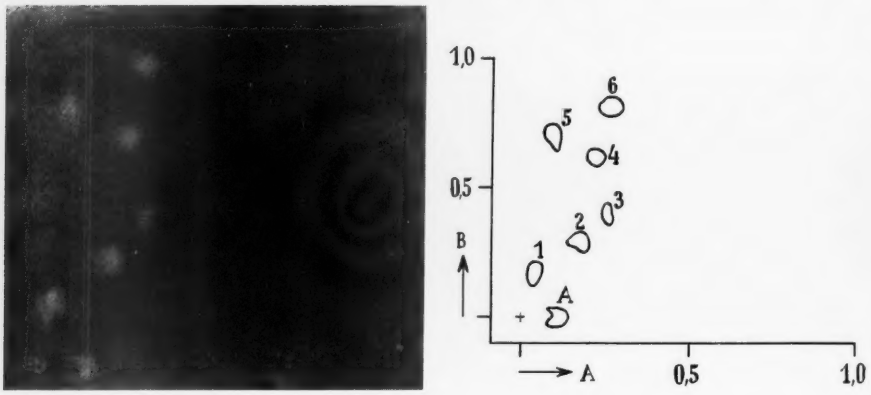
Tartaric acid was, on occasions, found. Oxalic acid was represented as a band and frequently the spot of uric acid was difficult to separate from it. Within the band of oxalic acid lie also the spots of phosphoric and of sulphuric acids. As chlorides are also dissolved out of the exchanger there was always a distinct spot corresponding to hydrochloric acid as well as one due to sulphuric acid.

Glucuronic acid has not yet been positively identified, although Nordmann and co-workers (13, 14, 15, 18) found it in adult urines.

Discussion

(a) *Acids of the citric acid cycle*

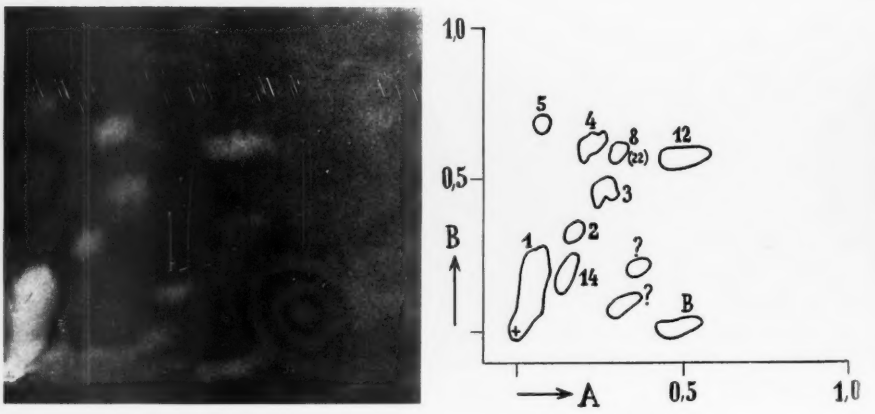
Before Nordmann's work only citric, α-keto glutaric and succinic acids had been found in urine. Nordmann *et al.* (16) were the first to find three more acids belonging to the citric acid cycle in adult urine; these were fumaric, malic and aconitic acids. The last and succinic acid were not regularly found. The chromatogram of a test solution of these six organic acids shows (Fig. 2) that they are distinctly



a. b.

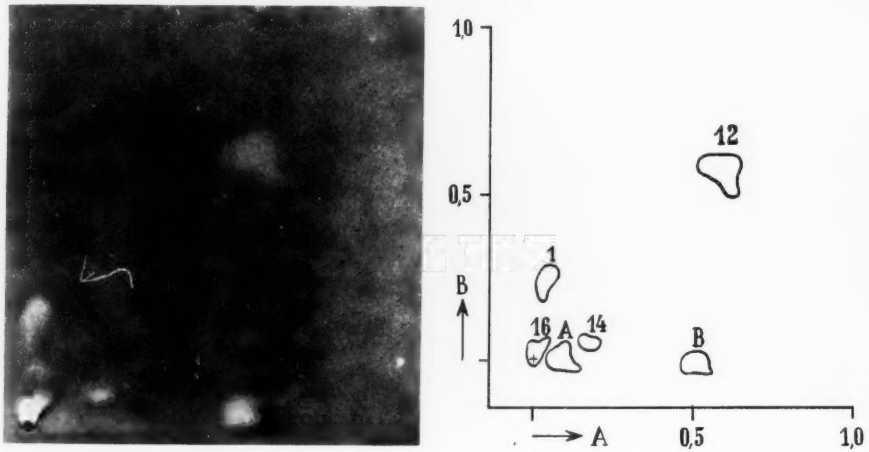
Fig. 2 a. Paper chromatogram of a test solution containing the acids of the citric acid cycle which are excreted in urine. Fig. 2 b. Identification diagram of the acids of the citric acid cycle. The quantities used for the test were as follows: 1. Citric acid (70 μg). 2. Malic acid (70 μg). 3. α-ketoglutaric acid (100 μg). 4. Succinic acid (70 μg). 5. Aconitic acid (70 μg). 6. Fumaric acid (70 μg). A. Sulphuric acid (30 μg).

Solvent A. Ethyl alcohol – NH_3–H_2O.
Solvent B. Propyl alcohol – Cineole – HCOOH–H_2O.
→Direction of the solvent run.
The material is placed on the cross in the left hand corner.



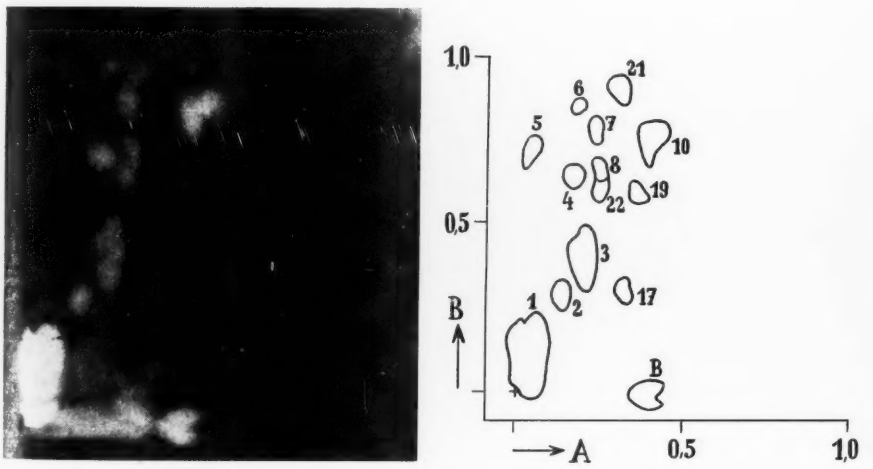
a. b.

Fig. 3 a. Paper chromatogram of foetal urine. Fig. 3 b. Identification diagram of organic acids found in foetal urine. The spots are numbered as in Fig. 1.



a. b.

Fig. 4 a. Paper chromatogram of serum from cord blood. Fig. 4 b. Identification diagram of organic acids found in serum from cord blood. The spots are numbered as in Fig. 1.



a. b.

Fig. 5 a. Paper chromatogram of urine passed on the 6th day of life. Fig. 5 b. Identification diagram of organic acids found in urine passed on the 6th day of life. The spots are numbered as in Fig. 1.

definable, and all six have regularly been found in the urines passed before and soon after birth. The missing acids of the citric acid cycle, namely oxalacetic and oxalsuccinic acids, are too unstable to be detected by the methods employed. These results indicate that acetylization and the citric acid cycle were in active operation before birth. Acetylization, which involves the participation of co-enzyme A, had already been shown to take place before birth by Hanon *et al.* (10) in quite another context.

The prominence of the spot due to citric acid is interesting in connection with the titratable acidity of the urine before and soon after birth.

(b) *Lactic acid*

Fig. 3 shows that a large spot corresponding to the position taken up by lactic acid was found in the chromatogram of foetal urine, and Fig. 4 that an identical spot was found in the chromatogram of cord blood. This lactic acid may be attributed to the partial anaerobic metabolism in the foetus (1, 5, 11, 20, 22) or to muscular exertion on the part of the mother during labour, followed by passage of the lactic acid so formed through the placenta (3, 7, 27), or it may be partly of foetal and partly of maternal origin (25). Large spots, due to lactic acid, however, were also found in all the urines passed on the first and second days of life, when respiration had been fully established. By the fourth day the spot attributable to lactic acid had become less conspicuous and it had disappeared by the sixth day (see Fig. 5).

Pyruvic acid was not detected but was probably present both in the serum and urine (9, 24). Owing to its unstable nature this acid does not lend itself to identification by paper chromatography (30).

(c) *General indications and comparisons with adults*

Differences in the regularity of occurrence of the acids in the citric acid cycle have already been mentioned. In adult urines Nordmann (15, 18) found that the aromatic acids predominated over the aliphatic ones, whereas the reverse has been the rule in this investigation. The explanation may be that the aromatic acids mostly originate in food, and particularly in vegetables. In the present chromatograms, moreover, glutaric, adipic and hippuric acid were regularly found. Nordmann and co-workers (18) only occasionally detected adipic and glutaric acid, but regularly found m-hydroxybenzoic acid, pyrrolidone carboxylic acid and phenyl acetyl glutamine. In accordance with the findings in adults, however, glycolic, β-hydroxybutyric and tartaric acids have only sometimes been found.

Summary

1. The organic acids in urine passed before and shortly after birth have been investigated by paper chromatography.

2. Eighteen acids were identified; six of them belonged to the citric acid cycle.

3. Lactic acid was prominent before and for a day or two after birth. It had become inconspicuous by the sixth day.

4. The aliphatic acids predominated over the aromatic ones. The reverse has been found by others in the urines passed by adults.

Acknowledgements

I am very grateful to Professor Jo Nordmann, Paris, for reporting on several paper chromatograms which were sent to him, and for all his valuable advice. I thank my teachers, Professor R. A. McCance and Dr. E. M. Widdowson, for all their support and encouragement, and I am indebted to Sister Elizabeth Colbourn for the care with which she collected the urine samples.

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Studies on Human Lactation

Relation Between the Dietary Intake of Lactating Women and the Chemical Composition of Milk with Regard to Principal and Certain Inorganic Constituents

by M. G. KARMARKAR and C. V. RAMAKRISHNAN

The quality of breast milk has long since been suspected to depend on the nutritional status of the lactating mother and several studies have been undertaken in the recent years to get more definite information on the relation between the dietary intake of the lactating mother and the composition of breast milk with regard to different constituents.

It is well known that the diet of the lactating mother in underdeveloped countries is generally deficient in regard to protein. The wide prevalence of Kwashiorkor among children in such countries (caused by extreme degrees of protein deficiency) gives rise to the suspicion that the protein content of breast milk in such cases may be generally inadequate. However, studies carried out on the relation between dietary protein and milk protein have been far from conclusive. While Deem, and Escudero & Pierangeli have reported a favourable effect of dietary protein on the protein content of milk, no relation between the two has been found by Hoobler, Kleiner, Tritsch & Graves, Ruzicic, Gunther & Stainer, and Gopalan.

Similarly inconclusive findings have been reported with regard to the fat content of human milk. Deem, Ruzicic, and Escudero & Pierangeli have reported a favourable effect of dietary fat on the fat content of milk, while Kleiner, Tritsch & Graves, and Gunther & Stainer have found no relation between the two.

Preliminary investigations carried out in this laboratory on the effects of the socioeconomic status of the lactating mother (which reflects the nutritional status of the mother) on the composition of milk are suggestive of a relation between these two variables (Karmarkar, Kapur, Deodhar & Ramakrishnan).

The conflicting findings reported in the studies cited above underline the need for a more systematic approach to the problem. Studies were therefore undertaken in order to investigate more thoroughly the relation between the dietary intake of the lactating mother and the composition of breast milk with regard to the principal constituents of human milk and the results are recorded in this paper.

Experimental

Materials and Methods. The subjects of this investigation were 60 lactating women of normal health living in and around Baroda and belonged to the lactation period of 3–4 months.

Collection of diet and its analysis for various constituents

The whole day's diet of the subjects was obtained by making collections of equal amounts of all the food stuffs consumed by the subject including snacks, beverages, and dietary supplements, if any, in the form of vitamins, minerals etc.

For analysis of food, separate weighing was made of each food-stuff and all the food-stuffs then thoroughly mixed and homogenised. A portion of the homogenised food was dried in an electric oven at 90°C for 12 hours.

To determine protein content of the dried food, the total nitrogen was estimated by the micro-Kjeldahl method and the value obtained multiplied by 6.25.

The fat and carbohydrate contents of food were estimated according to the method of Banerjee & Biswas.

A weighed amount of dried food was ashed in muffle furnace at 900°C for 12 hours. The ash was dissolved in a few drops of concentrated hydrochloric acid and the volume made up to 50 ml with glass distilled water. This solution was used for the determination of calcium, phosphorus and iron contents of food. The calcium content was determined according to the method of Shohl & Pedley, phosphorus content, according to that of Fiske & Subbarow, and iron content, according to that of Wong.

The collection of food taken and the analysis of same for the various constituents were carried out for three consecutive days for each subject and the average daily intake of the various constituents was therefrom estimated.

Sampling of milk for analysis

Samples for analysis were collected in all cases between two feeds at about 3 p.m. in the afternoon by voluntary expression. The samples were brought to the laboratory in thermosflasks containing ice powder and immediately taken up for analysis. The sampling was done on three consecutive days and the average value for the three samples was taken.

Estimation of fat and lactose contents of milk

The fat and lactose contents of milk were estimated according to the method of Chiba *et al.*

Estimation of protein content of milk

The protein content of milk was determined in the same way as that of diet except that the constant used for multiplying was 6.38.

Estimation of calcium, phosphorus and iron contents of milk

1.0 ml of milk was ashed in muffle furnace at 900°C for 6 hours. The ash was dissolved in a few drops of concentrated hydrochloric acid and the volume was made up to 25.0 ml with glass distilled water. This solution was used for the estimations of calcium, phosphorus and iron contents of milk which were made according to the methods indicated for analysis of diet.

Results

To find out the relation between dietary protein and protein content of milk, the data were first classified into a frequency distribution with regard to dietary protein, and the median and the two quartiles determined for the distribution. (The first quartile was 34.3 g, the median, 42.8 g, and the third quartile, 50.0 g.) The subjects were then divided into four groups according to the quartile range in which they fell. The mean protein content of milk for the subjects in each group was then determined.

TABLE 1. *Effect of dietary protein on the protein content of human milk.*

Classification of subjects	Number of subjects investigated	Mean protein intake per day g	Mean protein content of milk g/100 ml
Group 1. (Dietary protein 20.0 to 34.3 g)	15	26.650 ± 1.3360	1.216 ± 0.05151
Group 2. (Dietary protein 34.3 to 42.8 g)	18	39.130 ± 1.0960	1.394 ± 0.05990
Group 3. (Dietary protein 42.8 to 50.0 g)	12	46.890 ± 1.4600	1.414 ± 0.09420
Group 4. (Dietary protein 50.0 to 75.0 g)	15	62.420 ± 1.5640	1.475 ± 0.07760

TABLE 2. *Effect of dietary fat on fat content of human milk.*

Classification of subjects	Number of subjects investigated	Mean fat intake per day g	Mean fat content of milk g/100 ml
Group 1. (Dietary fat 8.0 to 27.5 g)	16	18.360 ± 1.5960	4.125 ± 0.2352
Group 2. (Dietary fat 27.5 to 50.0 g)	15	37.430 ± 1.3310	4.300 ± 0.1367
Group 3. (Dietary fat 50.0 to 72.0 g)	16	60.540 ± 1.6450	4.860 ± 0.2530
Group 4. (Dietary fat 72.0 to 115.0 g)	13	88.980 ± 6.730	4.716 ± 0.2766

TABLE 3. *Effect of dietary carbohydrate on the lactose content of human milk.*

Classification of subjects	Number of subjects investigated	Mean carbohydrate intake per day g	Mean lactose content of milk g/100 ml
Group 1. (Dietary carbohydrate 120.0 to 193.7 g)	15	165.08 ± 5.980	7.197 ± 0.0903
Group 2. (Dietary carbohydrate 193.7 to 219.6 g)	15	204.93 ± 8.132	6.945 ± 0.0665
Group 3. (Dietary carbohydrate 219.6 to 250.0 g)	17	230.26 ± 9.083	7.176 ± 0.05852
Group 4. (Dietary carbohydrate 250.0 to 381.0 g)	13	288.76 ± 6.878	7.307 ± 0.1552

Table 1 shows the relation between dietary protein and milk protein.

The data were again treated in a similar manner and the subjects classified into four groups with regard to dietary fat and the mean fat content of milk determined for each group. The results are shown in Table 2.

A similar reanalysis of the data with regard to the carbohydrate content of diet and milk yielded results presented in Table 3.

TABLE 4. *Effect of dietary calcium on the calcium content of human milk.*

Classification of subjects	Number of subjects investigated	Mean calcium intake per day g	Mean calcium content of milk g/100 ml
Group 1. (Dietary calcium 0.08 to 0.23 g)	17	0.1704 ± 0.01435	0.02048 ± 0.001414
Group 2. (Dietary calcium 0.23 to 0.40 g)	13	0.3279 ± 0.01526	0.02229 ± 0.001235
Group 3. (Dietary calcium 0.40 to 0.58 g)	14	0.4401 ± 0.05383	0.02029 ± 0.001475
Group 4. (Dietary calcium 0.58 to 1.90 g)	15	1.1443 ± 0.1386	0.02024 ± 0.001231

TABLE 5. *Effect of dietary phosphorus on the phosphorus content of human milk.*

Classification of subjects	Number of subjects investigated	Mean phosphorus intake per day g	Mean phosphorus content of milk g/100 ml
Group 1. (Dietary phosphorus 0.34 to 0.75 g)	14	0.5813 ± 0.03025	0.01137 ± 0.001100
Group 2. (Dietary phosphorus 0.75 to 0.92 g)	14	0.8205 ± 0.015665	0.00989 ± 0.001177
Group 3. (Dietary phosphorus 0.92 to 1.12 g)	16	1.0140 ± 0.02572	0.00929 ± 0.00071
Group 4. (Dietary phosphorus 1.12 to 1.90 g)	15	1.317 ± 0.0683	0.01177 ± 0.001298

TABLE 6. *Effect of dietary iron on the iron content of human milk.*

Classification of subjects	Number of subjects investigated	Mean iron intake per day mg	Mean iron content of milk mg/100 ml
Group 1. (Dietary iron 8.0 to 14.0 mg)	8	11.000 ± 0.2249	0.1928 ± 0.01449
Group 2. (Dietary iron 14.0 to 20.8 mg)	8	18.590 ± 0.5607	0.1723 ± 0.01630
Group 3. (Dietary iron 20.8 to 28.0 mg)	8	24.210 ± 1.132	0.2108 ± 0.03577
Group 4. (Dietary iron 28.0 to 40.0 mg)	8	33.230 ± 1.581	0.1885 ± 0.01736

Tables 4, 5 and 6 show similarly the relation between dietary intake and the constitution of milk with regard to calcium, phosphorus and iron.

It must be pointed out here that the four groups in the different tables are not identical and that the grouping is only with regard to the particular constituent dealt with.

Discussion

Table 1 shows a general increase in milk protein with dietary protein. The product moment correlation between the two was calculated (from the original data) and found to be statistically significant ($r = 0.3733$). It is further observed that the increase from Group 1 to Group 2 is statistically significant ($t = 2.253$, $p <$

0.05), while the increases beyond Group 2 are not so. This suggests that increases in dietary protein up to a certain level may have a favourable effect on milk protein, increases beyond this level having no effect. Such a suggestion appears to be capable of explaining the difference between the results obtained in this study and those reported by Gopalan, Gunther & Stainer and others. For it must be noted that the daily dietary protein of subjects used in the present study ranged from 20 g to 75 g, while the range reported by Gopalan is 60 g to 114 g and that reported by Gunther & Stainer is 76 g to 120 g.

It is seen from Table 2 that the first three groups show progressive increases in milk fat with dietary fat, the increase from Group 1 to Group 3 being statistically significant ($t = 2.128$, $p < 0.05$). No difference, however, is observed between Group 3 and 4 with regard to the fat content of milk despite the considerably greater fat intake in Group 4. It would again appear that initial increases in fat intake are accompanied by increases in the fat content of milk and that this relation does not hold beyond a certain level of dietary fat. The product moment correlation between dietary fat and milk fat (estimated from the original data) was found to be 0.7932.

The data presented in Tables 3, 4, 5 & 6 suggest, on the other hand, no consistent relation between dietary intake and the composition of milk with regard to carbohydrate, calcium, phosphorus and iron. The correlations between diet and milk contents were found to be negligible with regard to all these constituents.

Summary

The diet as well as the milk of 60 lactating women were analysed for protein, fat, carbohydrate, calcium, phosphorus, and iron contents. A significant relation was found between dietary protein and milk protein, and between dietary fat and milk fat.

Acknowledgement

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Staphylococcal Empyema in Infants

by KNUT BIE and JENS STEEN

An increase in hospital infections with penicillin-resistant staphylococci is apparent also in pediatric practice. In a maternity ward nearly 100 % of the newborn will have staphylococci in their nose, and the same strains can be isolated from about 50 % of the same infants six months after birth (5). In routine pediatric admission coagulase positive staphylococci have been isolated from nasopharynx in about 85 % of the children (3, 10). It is not surprising that staphylococcic skin infections are seen frequently in pediatric practice; patients with more serious staphylococcic infections, as osteomyelitis, empyema pleurae and septicemia, are admitted to the pediatric wards with increasing frequency.

In the Pediatric Department and the Surgical Department III, Ullevål Hospital, we have from September 1957 to May 1959 treated 8 patients with staphylococcal empyema pleurae. Six of the patients were between 14 days and 4 months of age—and two were 21 and 23 months old. The sex distribution was 1 girl and 7 boys.

The case histories are very similar. The infants had all been acutely ill for only few days prior to the hospitalization, most of them with high temperatures, coughing and signs of respiratory distress. In none of the children the diagnosis pleural empyema had been made prior to the admission. The source of the staphylococcic infection is unknown in the oldest child. A 21-month-old boy had a baby brother with pyodermia, and the six other infants were born within the last months in a municipal hospital maternity ward, during a period with frequent staphylococcal infections in the newborn and their mothers (pyodermia and mastitis).

The diagnosis was made clinically in the Pediatric Ward, and in all cases verified by X-ray examination, pleural puncture and bacteriologic examination of the pus. The diagnosis is definitely established when pathogenic staphylococcus aureus has been identified in culture from pleural fluid or from the blood. Positive cultures from nasopharynx do not establish the diagnosis with certainty. The antibiogrammes and the results of fage-typing (in five patients) are shown in Table 1.

The patients had a marked leucocytosis with white blood-counts up to 56,000 per cu.mm blood. A two-month-old boy however—who had symptoms of his disease only the day prior to the admission had a leucocyte count of 4300 per cu.mm.

The infants were extremely ill on admission, and treatment with parenteral penicillin, and with erythromycin or chloromycin was immediately started and continued after the bacteriologic confirmation of the diagnosis. High doses of crystalline penicillin have been given—about $\frac{1}{2}$ million units per kg/24 hrs for up to four weeks. Erythromycin and chloramphenicol were used in conventional doses, erythro-

TABLE 1. *Antibiogram and fage-typing of the staphylococci.*

Patient no.	1	2	3	4	5	6	7	8
Penicillin	0	0	0	0	0	0	2	0
Erythromycin	3	3	3	3	3	2	3	3
Tetracyclin	0	0	0	0	0	3	3	3
Streptomycin	3	0	0	0	0	3	3	2
Chloramphenicol	3	2	2	3	3	2	3	3
Sulfa	0	0	0	0	0	2	3	0
Fage-typing	—	—	—	80	80	29/52	3/55/711	80

mycin for up to five weeks, chloramphenicol never for more than two weeks. As a supplement cathomycin and sulfamethoxypyridazin were used on three of the patients. The first patient was treated in the Pediatric Ward with repeated pleural aspirations—every second day for two weeks. Seven patients were temporarily transferred to Surgical Department III where closed thoracotomy drainage was immediately instituted. The site for the thoracocentesis will depend upon the localization and extent of the empyema. Occasionally more than one area must be drained. A trocar is selected through which a No. 13–14 Nelaton catheter can be inserted. A small incision in the skin is made 1–2 cm below the intended penetration between the ribs, thus forming a flap to prevent air leak during the draining period (9). Additional openings should be made in the catheter before it is pushed through the lumen of the trocar and as far into the chest as desired. The catheter is removed as soon as the lung is expanded and the pus formation is negligible (Table 2). In our experience full expansion of the lung is not always immediately obtained, probably due to excessive fibrin formation and partly due to air-leak that may occur after some days of drainage. Because of residual air in the pleural space, drainage in some of our patients was continued for more than three weeks, but expansion of the lung was not complete in two of the patients until several weeks after the drainage had been removed. Klassen (7) advises decortication of the lung in such cases, but in patients in this early age group a great tendency towards spontaneous restitution can be expected,—major surgical intervention is rarely necessary (1). We have not used fibrinolytic enzymes intrapleurally, partly because of the expected reaction, and partly because of the risk of reopening a closed bronchopleural fistula. Local instillation of antibiotics has been done occasionally, and

TABLE 2. *Duration of treatment, fever and hospitalization.*

	Drug treatment	Thoracic drainage	Fever	Stay in hospital
No. of days (average)	38	19	16	49
No. of days (range)	28–45	7–42	5–30	38–72

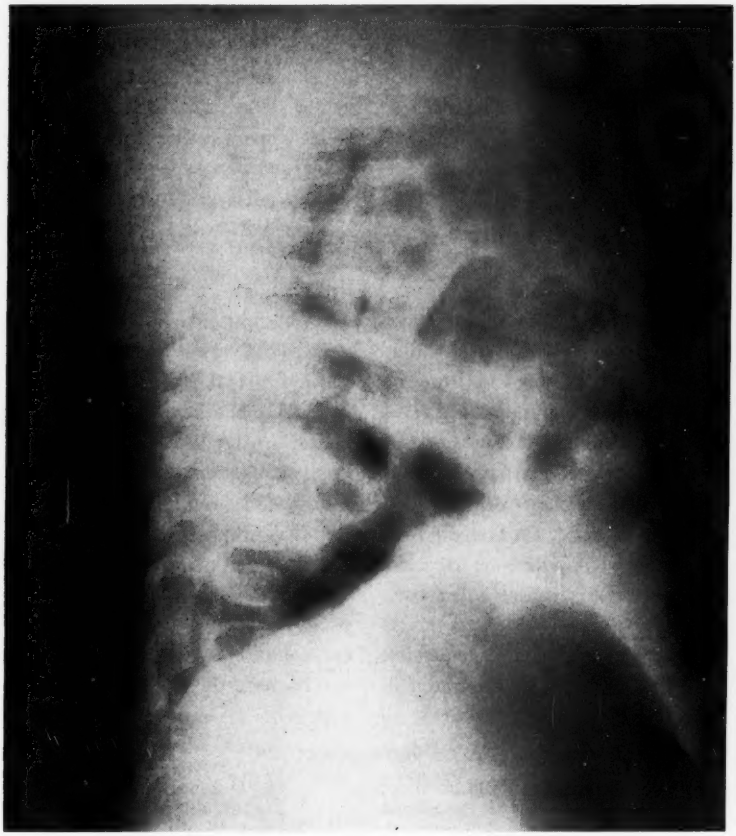

Fig. 1. Large pneumatoceles in a 3-month-old boy.

mostly in order to clear the catheter with a penicillin solution when the pus has been too thick to flow freely.

All of our 8 patients recovered completely from their disease. The pathological changes in the lungs disappeared gradually from the roentgenogrammes in the course of 2½–6 months.

The mortality rate of staphylococcal pneumonia before the antibiotics was high, and near 100 % in infants below 4 months of age (6). After the introduction of antibiotics the mortality rate has been considerably reduced, but remains relatively high in early infancy. Pryles (8) reports an overall mortality of 37.5 % among 24 children with staphylococcal pneumonia, and approximately 90 % of the deaths occurred in patients less than 4 months of age. Wallman (11) reports a mortality rate of 23 % in a series of 39 children. Harlem (4) has two deaths in 7 children with staphylococcal pneumonia.

Closed thoracotomy drainage in treat-

ment of the empyema has been used routinely in Surgical Department III, Ullevål Hospital since 1935, and the importance of this treatment has been pointed out by others (1, 2).

Staphylococcal pneumonia is known for its tendency to formation of multiple lung abscesses around small bronchi in the periphery of the lung. These necrotizing foci—not visible on the roentgenogrammes—may give rise to a broncho-pleural fistula and pyopneumothorax. A tension pneumothorax may develop with shift of the mediastinum toward the opposite side, and provoke a dangerous increase of the respiratory difficulties. Immediate treatment with closed drainage of the chest will give dramatic relief of the acute symptoms, as the lung will partly or fully re-expand, and the mediastinum will return to its normal position (2). Repeated simple aspirations from the affected pleura are usually not entirely successful and will often give only transient relief. Multiple pneumatoceles in the roentgenogrammes are characteristic for staphylococcal pneumonia (Fig. 1). These emphysematous bullae appear rather large, but do not as a rule require any special treatment, as they usually disappear spontaneously within four to eight weeks.

Summary

Eight patients from 14 days to 23 months of age have been treated for staphylococcal empyema with no deaths. The diagnosis has been verified bacteriologically after thoracocentesis in all cases. Early treatment with high doses of penicillin and erythromycin or chloramphenicol was instituted. Close cooperation between the pediatrician and the surgeon should be maintained, and the importance of prompt institution of closed thoracotomy drainage for acute tension pyopneumothorax is stressed.

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The Renal Tubular Reabsorption of Inorganic Phosphate in Primary Refractory Rickets

by R. STEENDIJK

Introduction

Primary refractory rickets is a form of late rickets characterized by several phenomena, the most important of which is the resistance to vitamin D therapy. Even in huge daily doses vitamin D can only establish an incomplete cure and the concentration of serum phosphorus remains subnormal.

The cause of this resistance is unknown and the pathogenesis of the disease has been the subject of some widely diverging concepts. The best-known theories are those of Albright and his co-workers and of Fanconi.

Albright, Butler & Bloomberg (1) believed the pathogenesis of infantile rickets and of primary refractory rickets to be similar. The low level of serum phosphorus was explained by increased activity of the parathyroid glands.

Fanconi (6, 7) postulated the existence of an intrinsic tubular defect in primary refractory rickets. This resulted in increased phosphate loss, which led to the low serum phosphorus level. Fanconi suggested the name "phosphate-diabetes" to denote this condition.

However, it appears from several balance studies that the balances of phosphate and of calcium in infantile and in primary refractory rickets are identically altered (1, 10, 11, 13, 19). With respect to phosphate intake, the renal excretion of phosphate has never been found to be significantly higher in primary refractory rickets than in infantile rickets (16). Moreover, Winberg & Zetterström (18, 19) noted that urinary phosphate excretion in primary refractory rickets depended on phosphate intake and could be either high or low without a change in the level of serum phosphorus. This casts doubt on the existence of a renal defect.

The studies to be reported were carried out to check Fanconi's concept. They resulted from the following arguments:

In normal individuals, the fractional tubular reabsorption of phosphate is high when phosphate intake is low, whereas it is low on a high phosphate intake. In this manner a constant level of inorganic phosphate in the blood is maintained (4, 9).

A normal renal tubular response to changes in oral phosphate load in patients with primary refractory rickets, would strongly suggest the absence of an intrinsic tubular defect.

Patients

The tubular reabsorption mechanism for phosphate was studied in 5 patients, suffering from primary refractory rickets: 2 boys, P.N. and P.V., respectively 14 and 16 years of age; and 3 girls, A.V., M.N. and P.S., respectively 12, 12 and 4 years of age. All children had received vitamin D in large doses for several months at least; radiographically active rickets was either absent or present in a very minor degree. The case records of these patients have been presented in detail elsewhere (15).

Methods

The fractional tubular reabsorption (TRP) was calculated from the simultaneous determination of the concentrations of inorganic phosphorus and of endogenous creatinine in serum and in urine.

If the clearance of creatinine is assumed to represent glomerular filtration rate:

$$\text{TRP}\ (\%) = \left(1 - \frac{\text{serum-creatinine} \times \text{urinary phosphate}}{\text{serum-phosphate} \times \text{urinary creatinine}}\right) \times 100$$

The concentration of serum phosphorus was determined according to the method of Briggs (2). The concentration of phosphorus in urine was determined by a modification of this method. Serum- and urine creatinine were estimated according to the method of de Vries & van Daatselaar (17). In this method only true serum creatinine is determined.

After a normal breakfast at 7.30 a.m., the patients took nothing by mouth except water. Between 11 a.m. and 12 noon they urinated and, following this, drank 100 to 200 ml of water. Half an hour later, venous blood was drawn for the estimation of inorganic phosphorus and creatinine. Another 30 minutes later, they urinated again. This urine specimen was kept for the estimation of phosphorus and creatinine.

The influence of diurnal changes in the concentration of serum phosphorus and in phosphate diuresis was avoided by limiting the collection of urine to one hour in the manner described.

To establish normal values of TRP with this method, this fraction was calculated in 12 children, 7 boys and 5 girls. These children were either healthy or had diseases in which there was no indication of a disturbance in the metabolism of phosphate or in kidney function. None of the control subjects were acutely ill and none received medication other than small

TABLE 1. *Calculation of the fractional reabsorption of phosphate in 12 control subjects.*

Subject	Age years	Diagnosis	Sex	Serum-creat. mg/l	Urine-creat. mg/l	Serum-P mg/l	Urine-P mg/l	P-clear / Creat.-clear.	TRP %
M.T.	11	bronchiectasis	M	4.6	460	50	810	0.162	83.8
B.K.	14	thrombocytopenia	M	6.2	1 400	48	1 680	0.155	84.5
P.B.	10	healed fracture	M	5.2	170	59	200	0.104	89.6
G.E.	12	healthy	M	5.9	180	46	150	0.107	89.3
H.E.	10	healthy	M	5.0	520	50	490	0.094	90.6
J.R.	15	haemophilia	M	6.6	1 250	49	400	0.043	95.7
J.E.	12	healthy	M	6.1	1 040	44	660	0.088	91.2
M.Z.	10	acute rheumatic fever	F	5.6	640	44	750	0.149	85.1
R.K.	10	bronchial asthma	F	5.9	710	52	980	0.157	84.3
L.P.	11	acute rheumatic fever	F	6.0	750	63	1 330	0.169	83.1
M.E.	10	patent ductus art.	F	4.6	940	50	670	0.065	93.5
I.K.	12	slight mucoviscidosis	F	4.3	320	52	470	0.122	87.8

TABLE 2. *Calculation of the fractional reabsorption of phosphate in 5 patients.*

Subject	Date	Therapy	Serum-creat. mg/l	Urine-creat. mg/l	Serum-P mg/l	Urine-P mg/l	P-clear. / Creat.-clear.	TRP %
P.N.	2– 9–'58	150.000 U. Vit. D/day	6.2	590	26	270	0.109	89.1
	16–12–'58	150.000 U. Vit. D/day	7.0	830	29	580	0.169	83.1
P.V.	21– 8–'58	150.000 U. Vit. D/day	6.5	1 490	36	920	0.111	88.9
	14– 2–'59	150.000 U. Vit. D/day	6.8	1 520	28	710	0.113	88.7
A.V.	15– 9–'58	120.000 U. Vit. D/day	4.9	1 410	31	1 100	0.123	87.7
	6–10–'58	120.000 U. Vit. D/day	5.3	125	39	170	0.185	81.5
M.M.	20– 8–'58	90.000 U. Vit. D/day	5.3	430	25	360	0.177	82.3
	21–10–'58	90.000 U. Vit. D/day	5.7	1 520	32	1 210	0.142	85.8
P.S.	2– 9–'58	75.000 U. Vit. D/day	3.9	410	38	880	0.220	78.0
	16–12–'58	75.000 U. Vit. D/day	3.6	200	28	300	0.251	74.9

amounts of sulfadiazine. The values found are presented in Table 1. The serum phosphorus concentration ranged from 44 to 63 mg/l; and TRP from 83.1 to 95.7 %. These values agreed well with those found by others in children from the age of 2 years (12, 14). Although one of our patients (P.S.) was much younger than the others, we omitted to determine the value of TRP for normal children of the same age, as a significant difference between these age-groups has never been found.

Results

The results of the calculation of TRP in the 5 patients are presented in Table 2. The estimations reported are those in which the highest and the lowest levels of serum phosphorus were found. These variations occurred in spite of adequate treatment and were unpredictable.

The range of the serum phosphorus concentration, 25 to 39 mg/l, was completely below that found in the controls. The range of TRP, 74.9 to 89.1 %, largely overlapped the normal range, but, on the whole, was lower. It is justified to conclude from these observations that the kidneys did not respond to the low level of serum phosphorus by an increase in TRP, even during intensive treatment.

To study the effect of additional oral phosphate on TRP, 3 children (A.V., M.M. and P.S.) were given a solution of the following composition:

Na_2HPO_4 2 aq.	4.6 g
NaH_2PO_4 2 aq.	1.0 g
aqua dest.	ad 60 ml

This mixture contains one gram of elementary phosphorus per 60 ml; the pH is approximately 7.3.

Fifteen ml of this solution were taken at breakfast. From that time the children fasted, but they took another 15 ml at 10.30 a.m. Urine was collected between 12 noon and 1 p.m., and blood was drawn at 12.30 p.m.

The results are shown in Table 3. TRP was substantially reduced in all 3 patients. A uniform effect on the serum phosphorus level was not found. A rise was observed in 2 patients (M.M. and P.S.): the level did not change in the third patient (A.V.). It

TABLE 3. *Effect of increase and decrease of intestinal absorption of phosphate on the fractional reabsorption of phosphate in 3 patients.*

Subject	Date	Therapy	Serum-creat. mg/l	Urine-creat. mg/l	Serum-P mg/l	Urine-P mg/l	P-clear. / Creat.-clear.	TRP %
A.V.	7–4–'59	60.000 U. Vit. D/day	5.5	1 450	34	1 220	0.136	86.4
	21–4–'59	60.000 U. Vit. D/day + addit. P.	5.6	850	34	1 690	0.328	67.2
	6–5–'59	60.000 U. Vit. D/day	5.8	1 560	30	1 660	0.206	79.4
	14–5–'59	60.000 U. Vit. D/day + $Al(OH)_3$	6.0	1 140	31	80	0.014	98.6
M.M.	24–3–'59	60.000 U. Vit. D/day	5.4	500	26	260	0.108	89.2
	6–4–'59	60.000 U. Vit. D/day + addit. P.	5.7	1 230	30	2 250	0.348	65.2
	18–9–'59	60.000 U. Vit. D/day + addit. P.	5.5	1 040	24	1 300	0.286	71.4
	25–9–'59	60.000 U. Vit. D/day + $Al(OH)_3$	5.5	640	24	100	0.036	96.4
P.S.	3–3–'59	75.000 U. Vit. D/day	3.8	390	31	770	0.242	75.8
	22–4–'59	75.000 U. Vit. D/day + addit. P.	4.0	290	37	1 290	0.481	51.9

must be mentioned, however, that the significance of this change in the serum phosphorus level cannot be evaluated, because the determinations with and without extra phosphate were not carried out on the same day.

In order to minimize intestinal absorption of phosphate, 2 patients (A.V. and M.M.) took 90 ml of a 5 % gel of aluminium hydroxide ("aluminox", Brocades & Stheeman, Amsterdam) per day, divided in 6 equal doses. Milk and milk-products were eliminated from the diet to reduce the dietary amount of phosphate as much as possible. Ingestion of aluminium hydroxide was continued until a few hours before TRP was determined. The results are also reported in Table 3. Phosphate excretion became minimal and TRP rose to very high values. The serum phosphorus level did not change.

The serum calcium level was stationary at a normal level throughout the investigations.

Discussion

The observations reported strongly suggest that an intrinsic tubular defect is absent in primary refractory rickets. Our findings support the results of the studies of Winberg & Zetterström (18, 19). They are also in agreement with the recently reported investigations by Dodge & Daeschner (5) and by Fields & Rice (8).

A renal action of parathyroid hormone is consistent with data presented. Chambers et al. (3) observed the effect of phosphate deprivation in patients with primary hyperparathyroidism. The serum phosphorus concentration did not change and TRP increased. Therefore, our observations are not in disagreement with Albright's concept of the pathogenesis of primary refractory rickets.

A direct action of vitamin D on the renal tubular reabsorption of phosphate, however, is doubtful in view of the normal response of the tubules to changes in phosphate load.

In normal conditions homeostatic factors, of which the kidney is the most important one in this respect, strive to maintain a constant level of serum phosphorus. The results of our studies indicate that, in primary refractory rickets, a similar homeo-

stasis exists at a lower concentration of serum phosphorus. The mechanism responsible for this is not known, although it is highly probable that the parathyroid glands are involved. Elsewhere we have presented further implications of this point of view, in connection with other types of rickets and with the modern theories of the biochemistry and biophysics of calcification (15).

Summary

Studies on the renal tubular reabsorption of phosphate in primary refractory rickets are presented. It is shown that:

1. In well-treated cases of primary refractory rickets, the fractional reabsorption of phosphate is low with respect to the serum phosphorus concentration,

2. Ingestion of additional phosphate is followed by a fall in the fractional reabsorption,

3. Phosphate deprivation is followed by a rise in the fractional reabsorption.

These results are in disagreement with the hypothesis that an intrinsic tubular defect is the underlying disorder in this disease. The findings are interpreted to signify a normal action of the tubular reabsorption mechanism, maintaining the serum phosphorus level at a low value, "set" by homeostatic factors, in which the parathyroid glands probably are involved.

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Analysis and Interpretation of the Fat-Absorption Coefficient

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Introduction

Steatorrhoea is often used as a criterion for the determination of the severity of absorption disturbances. In these disturbances also the influence of dieto- or drug therapy is judged by means of the steatorrhoea, the results being expressed in the fat absorption coefficient, viz.:

$$\frac{\text{g ingested fat–g excreted fat}}{\text{g ingested fat}} \times 100\ \% \quad (1)$$

For the interpretation of the absorption coefficient it is necessary to take into account many factors, such as the quantity and the kind of the ingested fat, the diet it is incorporated in, whether the patient has got used to a fixed quantity of fat or not, the rhythm of the defaecation, the length of the test period, psychic influences etc.

In this publication in particular attention is paid to the length of the test period in experimental investigations. Moreover consideration is given to some of the factors above in view of the interpretation of the absorption coefficient in normal persons as well as in patients.

1. Duration of the period of observation and calculation

a. *Moving average*

When in *normal* test subjects the absorption coefficient is daily determined and the values found represented in a graph, curves are obtained which show small oscillations (Fig. 1).

In normal test subjects these oscillations are mainly due to the fact that the amounts of faeces produced daily are not constant. These fluctuations, which are in no way related to the fat absorption, can be eliminated in the first place by inducing the test persons to defecate regularly, which is especially important in children, while in addition a 3-days' *moving average* of the absorption coefficient can be computed for every day (2). For example, for Wednesday this is the sum of the absorption coefficients of Tuesday, Wednesday and Thursday, divided by 3, for Thursday the sum of the absorption coefficients of Wednesday, Thursday and Friday divided by 3, etc. Just as in the intestine the chyme is subjected to a gradually proceeding process, the absorption coefficients merge gradually into one another when they are expressed in moving averages. When, for

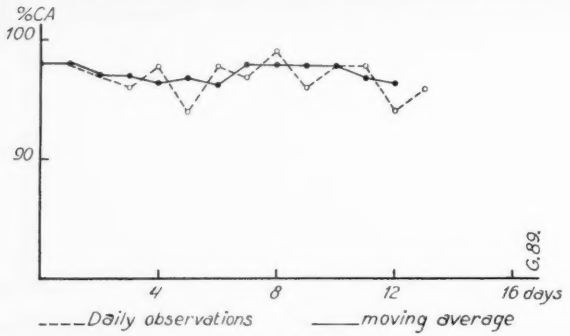


Fig. 1. Daily values and moving averages of the absorption coefficient of a normal test subject.

normal persons, the moving average of the absorption coefficient is plotted graphically, this indeed results in a practically straight horizontal line (Fig. 1).

The calculation of the moving average of the absorption coefficient is of particular importance in *patients* with steatorrhoea, as the deviations from the mean caused by the irregular filling and emptying of the colon are far more prominent in steatorrhoea because here more fat is excreted; it is, as it were, magnified projection of what is observed in normal persons. It is true that it is not quite impossible that variations in the fat absorption proper are overlooked to some extent by calculating the moving average, but this is more than offset by the certainty that the disturbing influence of non-essential fluctuations is reduced markedly, for in this way erroneous conclusions are prevented.

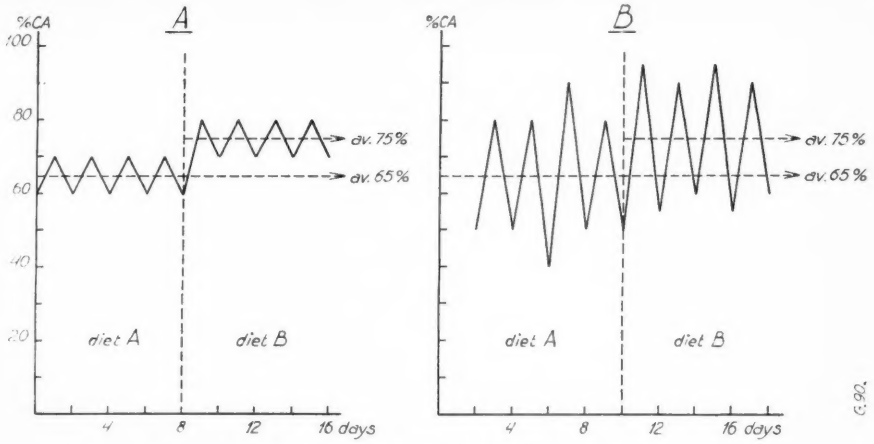
If, therefore, one wishes to obtain an idea of the severity and the course of a steatorrhoea, the 3-days' moving averages of the absorption coefficient must be determined for 24-hour periods over a certain number of days.

The moving average is mainly used to form an impression of the course of the steatorrhoea; this method is not used in the calculations discussed below.

b. *Mean CA*

When the absorption coefficient is determined daily to find out whether the patient suffers from steatorrhoea, this question can usually be answered after three days, while after five days it is possible to form an idea of the seriousness of the steatorrhoea. This impression will be more accurate the greater the number of observations. This greater accuracy is necessary for the investigation and comparison of the influence of different diets and drugs on the fat absorption.

When one wishes to arrive at the conclusion that the absorption coefficient has changed under the influence of a modification of the diet, and the *average* absorption coefficient for diet A is therefore compared with that for diet B, one must be certain that the difference observed between the average values is *significant*, so that the conclusion is justified that the therapy instituted has indeed led to the desired result. This is the more urgent because it is only rarely possible to test the influence of certain (dietary) changes several times in one and the same patient.



Figs. 2 A and 2 B. Influence of a change of diet on the absorption coefficient.

so that the single decisive experiment must be decisive indeed. Figs. 2 A and 2 B show how an absorption coefficient changes by a modification of the diet; Fig. 2 A for the case that the fluctuations are slight, Fig. 2 B for the case that they are considerable.

It is evident that in the first case (Fig. 2 A) also without further calculations the conclusion is allowed that there exists a significant difference between diet A and diet B. In the second case (Fig. 2 B) with the marked fluctuations, the difference will only be (statistically) significant when the number of observations has been great enough.

In other words, only by making a sufficient number of observations is it possible to arrive at reliable conclusions.

Here, however, we meet with a difficulty: how many observations form a 'sufficient number'? The answer to this question cannot be given off-hand, because this number depends on:

1. the fluctuations in the series of individual separate data;

2. the difference in absorption coefficient due to a measure taken, that one does not want to miss;

3. the chance one is willing to accept of making errors in the drawing of conclusions (i.e., to regard a non-existent difference as a real one or to overlook an existing difference);

4. the situation one is in with respect to the investigation (i.e., before, during or after the investigation).

c. *Number of observations*

It will not need further elucidation that the fluctuations in the separate data and the accuracy in drawing the conclusions has something to do with the number of observations to be made; we have, however, to enter in more detail into points 3 and 4, just mentioned which are also related with each other.

When a complete examination has been finished and the data of two series of observations of the absorption coefficient

are found, one series of the 'sick' period and the other of an immediately following period during which, for example, a 'curative' diet or some other measure has been applied, the number of observations is already fixed and one cannot do anything but find out whether the difference between the two average absorption coefficients can be called 'significant'. For this purpose, apart from this difference itself, its standard deviation is determined (in which both the fluctuation mentioned above and the numbers of observations play a role), and the difference is divided by its standard deviation. This division yields as quotient a figure which is indicated by the letter t in the mathematical statistics (hence the term t-test), and a chance value P corresponding with it can now be found in the t-table. This P indicates the chance one has to interpret the experiment carried out incorrectly, i.e., incorrect in the sense that the existence of a real difference is concluded to, while in reality the difference is only a fortuitous one. It is customary to conclude to an effect significant on the 5 % level when P values are read off equal to or smaller than 0.05 (equal to or lower than 5 %). When a chance is read off greater than 0.05, this indicates that the experiment carried out has no conclusive force enough; this situation can be the result of either a too small number of observations and then nothing can be done about it or of a too small or non-existent difference between the real absorption coefficients in the two periods.

How, then, is the situation when the whole experiment is still to be begun?

We may avail ourselves of the circumstance that there exists preliminary knowledge of the expected fluctuation (see below). Without this preliminary knowledge it would be difficult to give a more or less satisfactory answer to the question about the number of observations to be carried out, but with this knowledge a calculation can be made in which the requirements of points 2 and 3 are taken into account.

Let us try to form a picture of this situation. A patient, whose condition allows the time for a more or less accurate investigation, appears to yield such data in a 'sick' period of some days' duration that the real absorption coefficient may be assumed to be 80[1]; the physician decides to apply a certain curative diet and now wishes to know how long the whole investigation is to last.

As the lengths of both the 'sick' and the 'improved' periods are of influence for the standard deviation from the difference which is later to be calculated, it is logical to consider at the same time both the duration of the first period (the 'sick' period) and of the second period.

Based on his own experiences or on those of others, the physician will conceive an idea of the expected effect of his measure (or of the size of the effect which he is interested in) and he will for example assume that this measure will raise the real absorption coefficient to 90 (or he is only interested if the measure raises the real absorption coefficient at least to 90). This means an improvement of the real absorption coefficient by 10 points. He now wishes to set up his experiment in

[1] This, then, is the average of the population of absorption coefficients which would be found on continued investigation, when the patient's condition would remain constant for a protracted period.

such a way that, if this improvement of the real condition indeed occurs, he runs only a slight risk of having to conclude that this experiment does not yield a significant difference, after having carried out the whole experiment and after having analysed the figures according to the method described above (the *t*-test).

It is evident that this situation can very easily arise when it is realized that the two series of observations will be no more than relatively small samples and that they may yield results which differ considerably from reality; i.e., when the population of the absorption coefficients in the 'sick' period has a real average of 80, the average absorption coefficient over a limited number of days can very well happen to be 83, while the average of the second period may happen to be 87 instead of 90. The difference 87–83 would probably prove to be non-significant. A coincidence like this forms of course an 'unfortunate chance', but such an 'unfortunate chance' may arise all too easily in view of the wide fluctuations in the separate observations.

The situation before the experiment is therefore such that 'nothing is yet known', and that one has to safeguard oneself against the ill chance of doing a whole experiment for nothing. It is evident that in this way, before the experiment, a greater number of observations must be made than would appear to be necessary afterwards with the full knowledge of a completely finished experiment at one's disposal.

So far the general discussion of the problem: what is a sufficient number of observations? And now for the practice.

d. *Calculation of the number of observations*

It is self-evident that the *research-worker* has to use the 'official' mathematical methods when choosing the lengths of the investigation periods and in the determination of significances of differences between the periods. These methods require however a rather great amount of computation work and they are therefore less suitable for the practising physician. A method was therefore developed which, it is true, is somewhat inaccurate; this slight disadvantage is however more than counterbalanced by the great advantage that without intricate calculations one can directly read off from a table how experiments can be set up in a justifiable way. The result of our observations showed that there exists for coeliac patients a certain relationship between the mean absorption coefficient and its standard deviation. In Fig. 3 are plotted against each other the mean and the standard deviations of the absorption coefficients of all periods of 10 and more days of all our patients. It is clear that the standard deviation is lower, the higher the absorption coefficient, and conversely; it appears further that the points arrange themselves in a satisfactory way around the 'average' line which is drawn in. This graph is therefore so important because—with an accuracy sufficient for practical purposes—the value of the standard deviation on the drawn line can be read off from the mean absorption coefficient, so that the calculation of the standard deviations themselves is no longer necessary and only the simple calculation of the mean absorption coefficient is required. It is also possible to interpret this line statistically in such a way that the use of the formulas of the

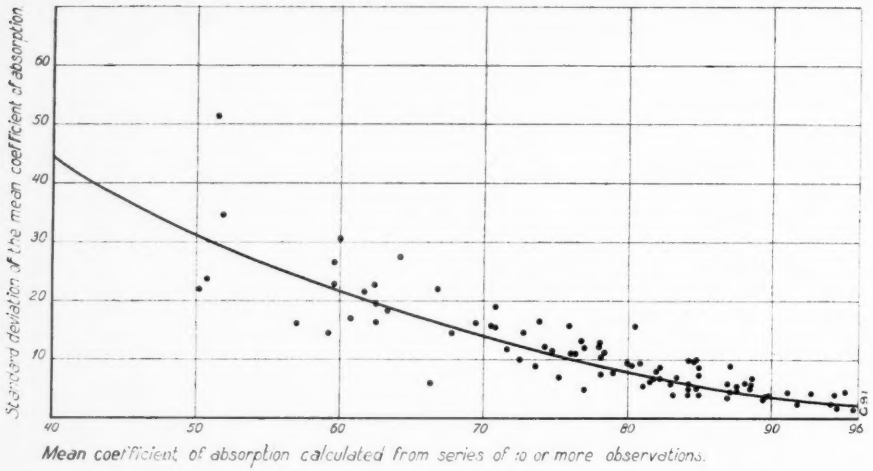


Fig. 3. Relationship between the mean and the standard deviation of the absorption coefficient for all our coeliac patients.

t-test, which are difficult to manipulate, can be abandoned and the formulas for the normal distribution can be applied.

All this in combination has created the possibility of drawing up some simple tables, which in most cases make calculation completely superfluous and in which everything can be found that is necessary to answer the following questions:

a. When we start from an estimation of the real absorption coefficient of a first period and from an expected improvement (or deterioration) during the second period: how long should both periods be taken in order that there is no greater chance than 5 % that later analysis of the figures leads to the conclusion that there is no significant difference, if the improvement was at least as large as expected.

b. Given an experiment carried out completely according to the indications obtained under point *a*: how great has been the probable minimum improvement (or deterioration), with a probability of 5 % of a lower figure?

c. Given a first period dealt with according to the indications obtained under point *a* and further, that the first days of the second period give the indication that the improvement (or deterioration) yields a figure higher than was expected: to what degree is it permitted to shorten the second period?

In Table 1, which is to be consulted when working in an ascending direction, the left column shows the value of the absorption coefficient in the first period. This column can be entered into with the average of the first period, in which it is allowed to interpolate linearly, rounding off in an upward direction. On top of the columns the improvement expected in the real absorption coefficient is shown. The table gives the number of observations to be carried out in the two periods under comparison, in order to run a chance not

TABLE 1. *Number of observations required in the test periods under comparison to demonstrate significantly an improvement of the mean fat absorption, which is at least as large as the expected improvement.*

Absorption coefficient in % in the first period	Expected improvement of the mean fat absorption								
	+5	+10	+15	+20	+25	+30	+35	+40	+45
90	13								
85	25	5							
80	47	10	5						
75	84	17	7	5					
70	143	31	12	6	5				
65	231	48	19	10	6	5			
60	340	73	28	15	9	6	5		
55	493	108	43	22	13	9	6	5	
50	709	154	62	32	19	13	9	7	5

exceeding 5 % that after the end of the experiment the difference found cannot be considered significant (if the 'real' improvement is at least equal to the expected improvement!).

Examples

a. Suppose that the examination during 10 days of the first period (in which at least 10 daily observations of the absorption coefficient must have been made, see Fig. 3) has yielded the average figure of 75. Suppose further that such a measure will be taken that an improvement of 10 points in the real value of the absorption coefficient is expected. The table is entered into with 75 and with +10 and 17 days are read off. The first period must therefore be prolonged to 17 days and the same must be done with the second period.

b. Suppose that the two periods have been dealt with according to the prescriptions and that they have yielded absorption coefficients of 76 and 87, respectively. How great was then the real improvement at least? The probable value of the improvement is 11, while it is at least equal to the difference between the two values found minus half of the improvement expected in the beginning. Thus:

$$87 - 76 - \tfrac{1}{2} \times 10 = 11 - 5 = 6$$

There is only a 5 % chance that the improvement is less than 6 points of the absorption coefficient.

c. Suppose that the first period has been dealt with according to the prescriptions and that an absorption coefficient of 76 has been obtained. The first five days of the second period yield the figure of 92. Evidently an error has been made in the expectation and now it seems reasonable that the second period—as fixed beforehand—need not be observed in full. How far can this second period be shortened? Once again the table is entered into, now with 75 and +15, and a duration of 7 days is found. Properly speaking, the first period has been taken too long, and nothing can be changed in this respect.

TABLE 2. *Number of observations necessary in the test periods under comparison to demonstrate significantly a certain decrease of the mean fat absorption*

Absorption coefficient in % in the first period	Expected decrease of the mean fat absorption								
	−5	−10	−15	−20	−25	−30	−35	−40	−45
95	13	5	5	5	5	5	5	5	5
90	25	10	7	6	6	6	6	7	
85	47	17	12	10	9	9	9		
80	84	31	19	15	13	13			
75	143	48	28	22	19				
70	231	73	43	32					
65	340	108	62						
60	493	154							
55	709								

On the other hand, it is now permitted to reduce the second period to 7 days.

This was a case in which the improvement was *more* than expected. Also when during the second period the improvement appears to be *equal* to the expectation, this period can be shortened. This reduction may go as far as to one-fifth of the original read-off duration, with a minimum of five days. In other words, when during the after-period it appears that a value of 85 is reached indeed, this period may be shortened to five days. However, after this curtailing the conclusion does not go further than that there has been an improvement, without indicating the extent of this improvement.

Table 2 should be used for working in a descending direction. The explanation of this table is the same as for Table 1, it being understood that this table refers to deterioration, and *that here it is not allowed to shorten second periods.*

There is finally a third table which should be consulted after the end of the whole investigation.

As set forth above, the Tables 1 and 2 and the possiblity of omitting the normal mathematical calculation methods are i.a. based on the circumstance that use can be made of the preliminary knowledge of the fluctuations as laid down in Fig. 3. However, this also implies that the fluctuations in the collected series of observations (which must be composed of day-

TABLE 3. *The permissible 'range' (= difference between highest and lowest observation) in a series of n observations; chance 95 %*

Number of observations	Mean absorption coefficient					
	95	90	85	80	75	70 and lower
2	8.3	10.2	16.6	23.5	30.5	40.2
3	10.0	15.0	20.0	28.4	36.7	48.4
4	11.0	16.4	21.9	31.0	40.2	52.9
5	11.6	17.4	23.2	32.9	42.6	56.1
6	12.1	18.2	24.2	34.3	44.4	58.6
7	12.5	18.8	25.1	35.5	46.0	60.6
8	12.9	19.3	25.7	36.5	47.2	62.2
9	13.2	19.8	26.3	37.3	48.3	63.7
10	13.4	20.2	26.9	38.1	49.3	65.0
15	14.4	21.6	28.7	40.7	52.7	69.5
20	15.0	22.5	30.1	42.6	55.1	72.6
30	15.9	23.9	31.8	45.1	58.1	76.9
50	16.9	25.4	33.8	47.9	62.0	81.8

observations) do not deviate too markedly from those of Fig. 3. This should be checked, which can be done by determining the ranges of each of the two series —viz. the range in the first period and the range in the second period—and finding out whether both answer the requirements of Table 3. (As said before, the 'range' is the difference between the highest and the lowest observation of a series.) Should this not be the case, in other words, is one of the ranges greater than the value shown in the table, the fluctuation in the collected series is non-permissibly greater than the values which formed the basis of Tables 1 and 2, and then these tables can no longer be used. In this case the 'official' mathematical methods should be used, for which, if necessary, a statistician should be contacted.

2. Interpretation of the C.A

a. *Normal fat absorption*

An absorption coefficient of 100 % is never found. From this it follows that either not all the fat is absorbed, or that always a certain amount of fat is excreted. In normal persons, however, it is practically generally accepted that the faecal fat is of endogenous origin. A coefficient of 95 % or more is normal for normal adults, children and toddlers older than 18 months. The absorption coefficient is 80–90 % for normal infants younger than 9–10 months, and for prematures this value can be even much lower (Fig. 4).

These values hold only good when the diet contains sufficient fat and when the fat which is absorbed is butterfat, vegetable oils and fat with low melting point. When much fat from sheep, cows or pigs is used, more fat is recovered from the faeces (in normal persons!) so that the absorption coefficients amount to 90–95 %.

If the fat content of the diet is below a minimum level (for infants of 0–1 years below 15 g, for toddlers below 20 g), low absorption coefficients are calculated. This is simply a result of the fact that the influence of the endogenous fat on the calculation of the absorption coefficient increases to a very great extent when the amount of fat taken up with the diet is low. Therefore it is not allowed to conclude in this case that the absorption of fat is disturbed.

b. *Disturbed fat absorption*

A normal absorption coefficient does not always imply that the absorption is normal in all respects.

This is due to the fact that the absorption coefficient is a criterion for the absorption of *all* fatty acids present in the alimentary fat. In various absorption disturbances, however, the different fatty acids are absorbed in a very different degree. As, for example, olive oil consists mainly of the unsaturated oleic acid while butter contains much saturated fatty acids, the absorption coefficient in the first case is mainly a criterion for the absorption of oleic acid, in the second case for the absorption of saturated fatty acids. This can be demonstrated in coeliac disease children. If in their diet butterfat is replaced by olive oil the absorption coefficient can be seen to rise suddenly from 85 % to 95 %, the normal value (Fig. 5). In spite of the fact that the steatorrhoea has disappeared, the conclusion is not allowed that

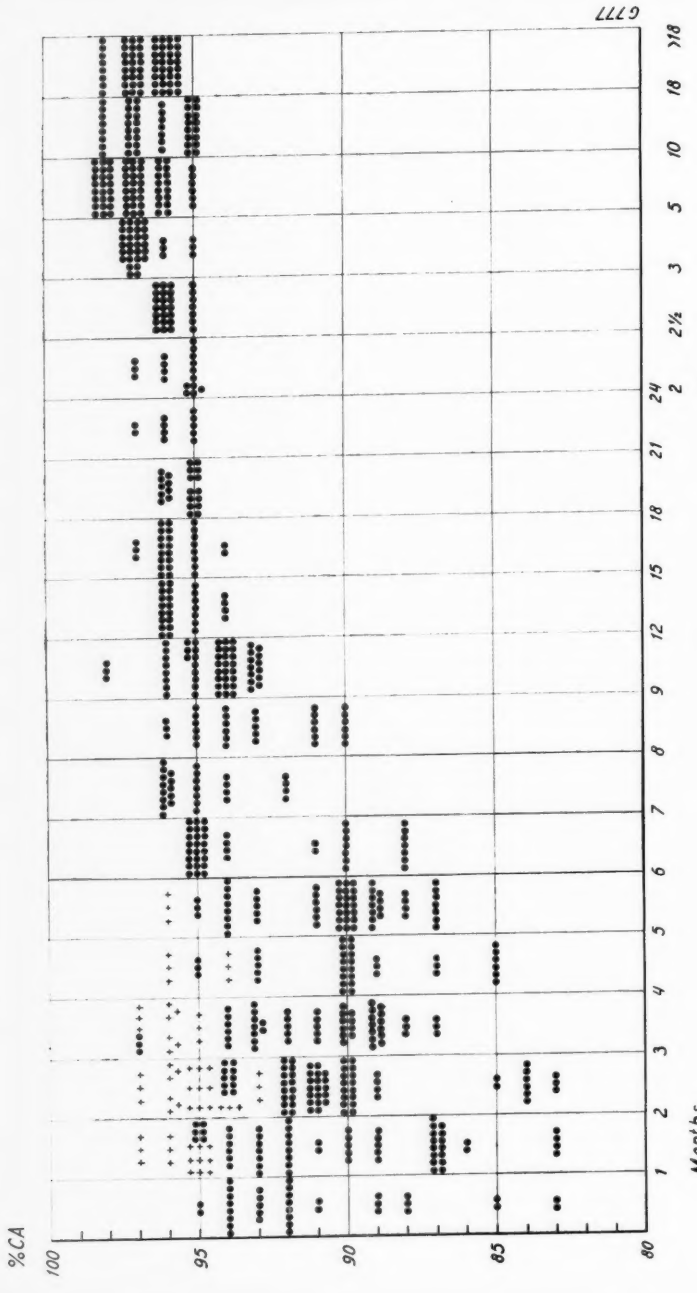


Fig. 4. Absorption coefficient of *normal* infants, pre-school children and adults.

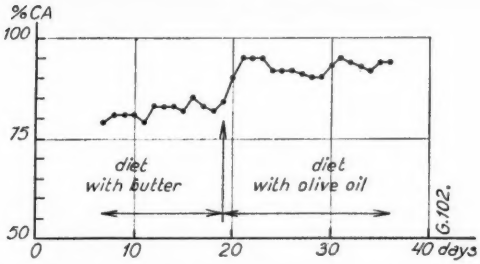


Fig. 5. Increase of absorption coefficient following change in fatcomponent of diet.

the overall fat absorption is now also normal, as the absorption coefficient falls directly again when administration of butterfat is resumed. As in general in patients with steatorrhoea oleic acid is much better absorbed than the saturated fatty acids, it is understandable that the absorption coefficient can rise from 85 % up to 95 % without the underlying disturbance being affected (3).

Apart from the special case mentioned just now, the mean value of the absorption coefficient over a given period shows whether the fat metabolism is seriously disturbed or not. More cannot be deduced from it. When, however, the daily values from which the mean absorption coefficient has been calculated, are taken into consideration, it becomes clear that we have an additional fact at our disposal. An average of for example 75 % can be composed of values varying between 60 and 90 %, and just as well of values between 70 and 80 %. We have found that the extent of the fluctuations forms a valuable criterion for the severity of the disease (for example coeliac disease and pancreatic fibrosis). Large fluctuations point to a much more serious condition than smaller ones. This observation proves to be of importance for practical purposes, as we always saw that a child with a markedly fluctuating absorption coefficient—but with a relatively good mean value (e.g. 80 %)—responded very unfavourably to infections, dietary changes, etc. In this way one has, in addition to the clinical observation, an objective criterion at one's disposal to decide whether—in in spite of a relatively good fat absorption coefficient—a coeliac disease patient is in a labile condition, which is very important from a therapeutic point of view.

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A Study of the Enzyme Pattern in Children and Newborn Infants

by GUNNAR CHRISTIANSSON and BERTIL JOSEPHSON

Comparatively little is known about the development of the concentration of the plasma enzymes in children's blood, even of those enzymes which are determined routinely in clinical practice. Kove, Goldstein & Wróblewsky (6) and Stave (11) studied the concentration of serum-glutamic-oxaloacetic acid transaminase (SGO-T) and serum-glutamic-pyruvic acid transaminase (SGP-T) in plasma from newborn infants. The alkaline phosphatase in children's serum has been studied by von Sydow (13). It is, however, well known that in children the composition of the blood plasma is not identical with that of adults, because the average concentration of several substances of diagnostic interest is different from the corresponding average value in blood from adult man (4).

A knowledge of the enzyme concentrations and of the development of the enzyme pattern from birth would be of clinical and ontogenetic interest. A comparison between the enzyme pattern in the plasma from the newborn and the pattern in the mother's plasma would be of physiological importance.

In the present investigation SGO-T, SGP-T, lactic acid dehydrogenase (LD) and serum alkaline phosphatase were determined in the same samples of blood from newborn infants and from a number of children aged one day to seven years. A comparison was also carried out between the enzyme activities in the plasma of a number of mothers and the corresponding activities in plasma from their newborn children. In the newborn children the serum bilirubin concentration also was determined because of the suspicion that their enzyme concentrations could be more or less related to their liver function and to the presence or absence of an icterus neonatorum (1). A preliminary note about some of our results has been published (2).

Material

All the children examined were from nurseries belonging to the city of Stockholm or from the Obstetric Clinic of St. Erik's Hospital, Stockholm. The age distribution is reported in Table 1. All the children were routinely examined by an experienced pediatrician and samples were taken only from children with no sign of disease. Samp-

[1] The investigation was supported by a grant from the Foundation "Thérèse och Johan Anderssons Minne".

TABLE 1. *Age distribution of the children included in the material.*

Age	Days							Months					Years						
	0	1	2	3	4	5	6	2	4	6	8	10	1	2	3	4	5	6	7
a) SGO–T	36	11	16	16	9	3	2	3	4	5	4	4	1	4	5	8	17	8	7
b) SGP–T		5	8	7	2	1													
c) LD		6	9	9	5														
d) Alkaline phosphatase	38	13	14	13	9	2	2	6	4	5	6	4	3	4	5	8	21	10	7

les from the newborn infants were taken only if both mother and child were considered healthy and if the delivery was normal.

From the newborn infants the samples were taken directly from the umbilical cord carefully avoiding any admixture with amniotic fluid. From the older children the blood was taken by venipuncture from a femoral or a brachial vein. The mother's blood was always taken from a brachial vein as soon as possible after delivery. The serum samples were discarded if any trace of hemolysis was visible.

The samples were immediately brought to the laboratory and centrifugated. The analysis was carried out the same or the following day. In the latter case the serum was stored at a temperature of about + 4°C.

Methods

Serum-glutamic-oxalacetic transaminase (SGO-T)

Karmen (5) as modified by Ordell (8). In Ordell's modification the number of pipettings is reduced by mixing before use: the aspartic acid, the DPNH, the malinic acid dehydrogenase—and the buffer solutions. As buffer solution THAM from Sigma was used and EDTA added. The final volume of the solution to be read is 3.6 ml instead of 3 ml in the original method. The procedure gives values in accordance with the original method.

Serum-glutamic-pyruvic acid transaminase (SGP-T)

Wróblewski & La Due (14) as modified by Ordell (unpublished). In this modification the corresponding simplifications as for SGO-T are introduced. The results are in accordance with the original method.

Lactic acid dehydrogenase (LD)

Wróblewski & La Due (14).

Alkaline phosphatase

Sommer (9).

Bilirubin

Jendrassik & Gróf (3) modified for 0.1 ml serum and Beckman B photometer.

Results

The determined mean values and the standard deviations for SGO-T and phosphatase are illustrated in Figures 1–2. In calculation of the means for SGO-T three extremely high values were excluded. It was felt that this would give a mean value which corresponded more correctly to the normal enzymatic pattern than if these values had been included. These values were: 3 days–120 units, 1 day–167 units, 3 days–125 units. One of these serum samples was icteric. These children were examined again three months later and appeared perfectly healthy. New blood-samples taken at this subsequent examination showed enzyme values within the normal range. For the same reason one extremely high value for SGP-T was excluded (400 units).

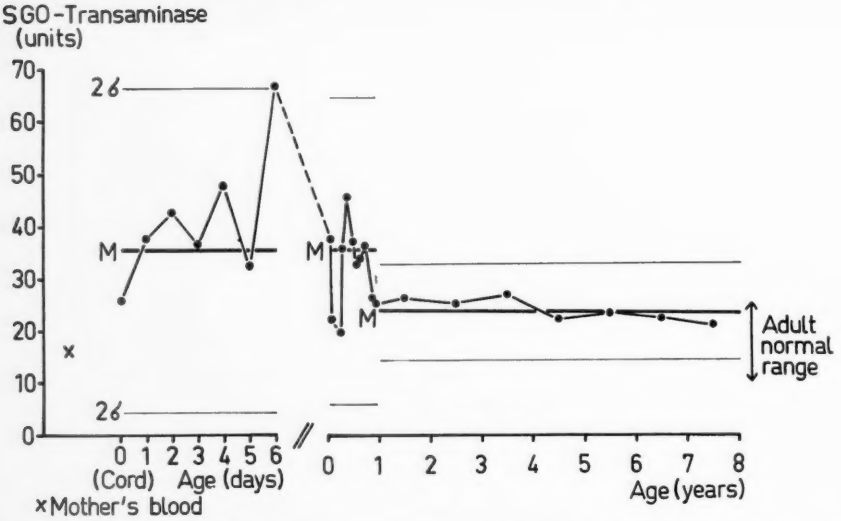

Fig. 1. Serum-glutamic-oxalacetic acid transaminase (SGO-T) activity in children of different age. Each dot represents the mean value from a group of children aged according to the abscisse. The number of children in each group is given in Table 1. The horizontal line represents the mean values and twice the standard deviation for the groups aged 0–6 days, 1–12 months and 1–7 years respectively. Three very high values are omitted (see text).

For SGP-T and LD the material was too small for calculation of mean values. For these enzymes all the values are recorded in Figures 3 and 4.

The serum bilirubin concentration in the newborn children varied between 0 and 14 mg per 100 ml. As there was no correlation between the bilirubin concentration and that of the determined enzymes the bilirubin figures are not given or plotted.

Discussion

SGO-T (Fig. 1)

The mean value for SGO-T in newborn infants, 35 units, was considerably higher than in adult man. The mean values for the small groups of children aged one week to one year were very varying, but for all these groups together the common mean value was identical with that of the newborn infants. The standard deviation for children aged less than one year was much higher than for older children and adults.

The mean value for the mothers was identical with the normal value for adults and thus significantly lower than the figure for the newborn children.

For children 1 to 7 years of age the mean value was 23 units which is somewhat higher than the normal adult value. The difference is not statistically significant. For these groups the value was slightly decreasing with age towards the adult mean, but the groups were small and the differences are not significant.

It does not seem probable that the high values in the newborn infants are a sign of liver dysfunction because usually parenchymatous liver disease gives much higher

values. It seems more probable that these high values are related to the increased erythrocytolysis in early childhood. This explanation cannot be valid for the high values encountered in some of the groups between 1 week and 1 year old. As these groups are small and the values very varying, there is scarcely any reason to try to find an explanation.

From a diagnostic point of view it is suggested that in children up to 1 week of age a SGO-T value of up to 70 units might be considered as normal. For children 1 week to 1 year old the normal values seem to be below 50. However, values between 50 and 70 cannot be definitely considered as pathological while the upper limit in children up to seven years must be set to 40 units.

SGP-T (Fig. 2)

The material consists of newborn children only and is too small for a statistical analysis. Except for one single value which was very high, the figures are evenly distributed below 50 units. This figure may be considered as the upper normal limit for newborn infants. It is significantly higher than the corresponding value for adults (30 units). These values also agree with those found by Wróblewsky et al (14) in newborn infants.

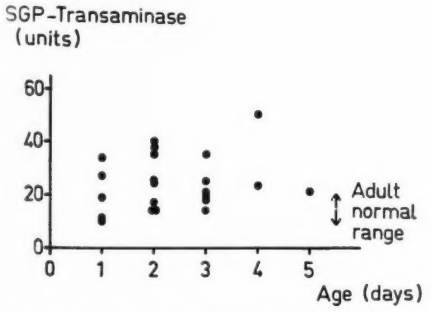

Fig. 2. Serum-glutamic-pyruvic acid transaminase (SGP-T) activities in relation to age. One extremely high value is omitted.

LD (Fig. 3)

This material also consists only of newborn children and is too small for statistical treatment. Except for three very high values all the figures are within or slightly above the normal adult distribution range (260–850 units). It seems as if values up to 1050 units could be considered as normal during the first week of life.

Alkaline phosphatase (Fig. 4)

It turned out that during the first week of life the alkaline phosphatase concentration was similar to that in adults (0–10 units) and with the same standard deviation. This had been observed earlier (13). It has been found that the alkaline phosphatase activity in the serum of

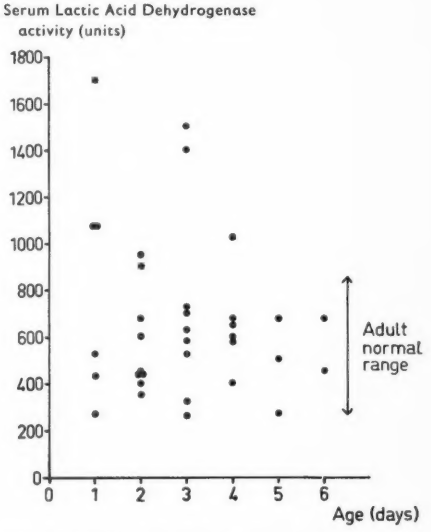

Fig. 3. Lactic acid-dehydrogenase activities in relation to age.

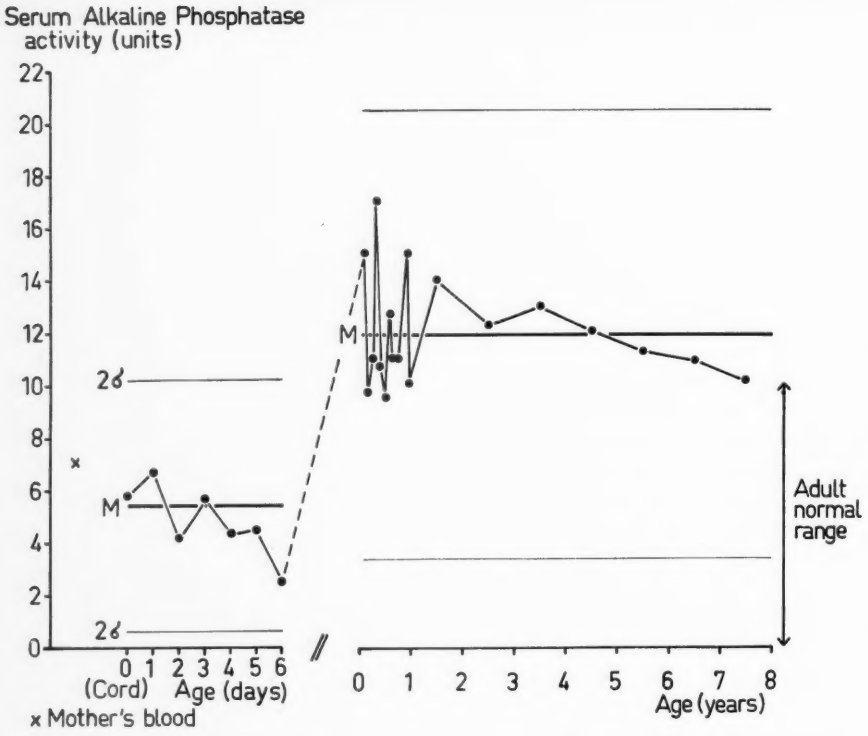

Fig. 4. Alkaline serum phosphatase activity in children of different age. Legend as in Fig. 1.

pregnant women increases towards term (10) which possibly might be connected with the increased production of estrogenic hormones. In the present material the serum phosphatase activity was significantly lower in the newborn infants than in their mothers.

Already at the age of one month the phosphatase value had increased to a mean of 12 units which is significantly higher than the adult value, and also the standard deviation was significantly higher than in adults. The value started decreasing slowly at the age of five years but at the age of seven, it was still significantly above that of adults.

It seems probable that the high phosphatase activity in children is connected with the development of the bone tissue. However, the ossification process is very intense precisely during the first week after birth and consequently high values could be expected also during this period. The reason for the low values found is obscure.

The same values as for adults are suggested as limits for the phosphatase value during the first week of life, i.e. 0–10

units. For the age one month to seven years the upper limit was found to be 20 units.

The enzyme pattern

Generally, it seems as if the enzyme pattern in the plasma of newborn infants differs considerably from that of adult man. This may be considered as a sign of immaturity rather than a sign of disturbed liver function as there was no correlation between the elevation of the different enzymes nor between the enzyme concentration and the degree of jaundice. Neither did a high enzyme value have any bearing on the health and further development of the child. The high standard deviation in the newborn children is in accordance with the hypothesis that the increased concentrations depend on the fact that the enzyme pattern is under development.

However, the strange exception represented by the alkaline serum phosphatase remains unexplained. A possible explanation would be that the increased values observed during the subsequent childhood might be due to the presence in plasma of some substance activating the phosphatase as postulated in certain cases by Thannhauser, Reichel, Grattan & Maddock (12). If this were the correct explanation the activating substance would not appear in the plasma during the first week after birth.

Summary

The concentration in serum of some enzymes often determined for diagnostic purpose was studied in children of different ages.

Serum glutamic-oxalacetic acid transaminase (SGO-T) activity was considerably higher during the first week of life than in adult man and so was the standard deviation. After one year it dropped to values near the normal value for adults.

Serum glutamic-pyruvic acid transaminase (SGP-T) was studied in newborn children only. The mean value and the standard deviation were considerably higher than the corresponding value in adults.

Lactic acid dehydrogenase (LD) was also studied only in children aged up to one week. The values were within or slightly above the range for normal adults.

Alkaline phosphatase activity was found to be within the normal range for adults during the first week after birth. Subsequently and up to the age of seven years the mean value and the standard deviation were significantly higher than in adult man.

The serum enzyme values from the newborn children were compared with the corresponding values from their mother's blood.

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The Uric Acid Concentration in Serum from Children, Newborn Infants and Mothers after Delivery

by GUNNAR CHRISTIANSSON and BERTIL JOSEPHSON

Studies of the development of the chemical pattern of children's serum have been previously published from these departments (1, 2, 4, 5) and it was supposed that such investigations are of physiologic, ontogenetic and clinical interest.

One serum constituent which has not been studied in children of different age is uric acid.

Material

The same blood sample as in the previous paper on enzyme pattern of children's plasma were used. The number of children examined is recorded in Table 1.

Method

The enzymatic method of Kalchar and Praetorius as modified by Fechtmeir & Wrenn (3).

Results

The results are demonstrated in Fig. 1.

Comments

During the first week of life the concentration of uric acid in the serum was significantly higher than in normal adults and so was the standard variation. However, it was found that the concentration value from the mother's sera was equally elevated. Thus it seems likely that uric acid from the mother's blood was in equilibirium with that of the fetus over the placenta. It is known that amniotic fluid has a higher uric acid concentration than serum. The high concentration in the mother's blood might be related to an increased formation and metabolism of nucleotides.

In children aged more than one year the concentration was not significantly different from that in adult man.

TABLE 1. *Age distribution of the children included in the material.*

	Days							Months					Years						
Age	0	1	2	3	4	5	6	2	4	6	8	10	1	2	3	4	5	6	7
Number	37	19	5	6	3			5	4	5	6	5	3	6	7	5	19	10	7

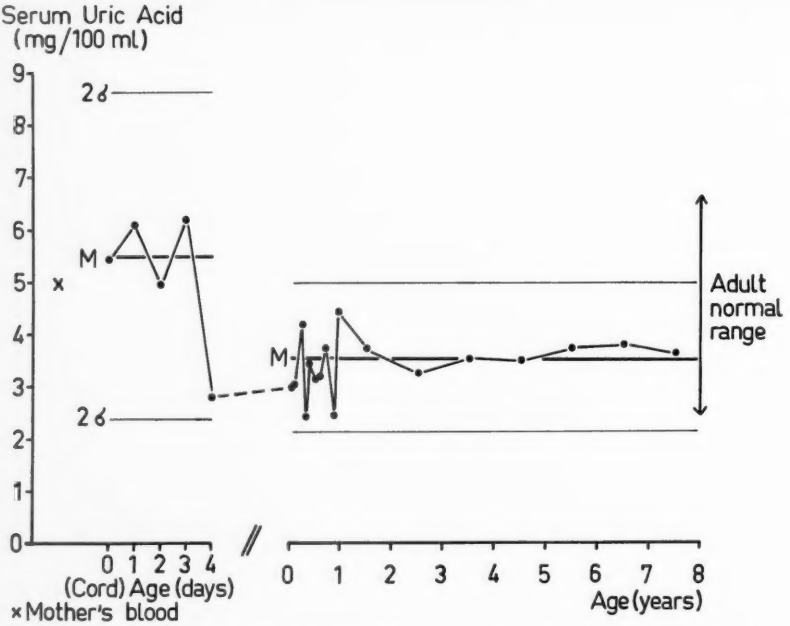

Fig. 1. Mean values and twice the standard deviation for serum uric acid in children in relation to age and in the mothers in relation to their newborn infants.

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CASE REPORTS

Two Cases of Aminoaciduria, Ocular Changes and Retarded Mental and Somatic Development (Lowe's Syndrome)

by ERIK TERSLEV

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(Chief: Professor Preben Plum, M.D.)

In 1952, Lowe, Terrey & MacLachlan in Boston described three cases of a syndrome in children which seemed to form an entity not previously described. The syndrome presented the following main symptoms: mental retardation, acidosis, hydrophthalmus, aminoaciduria, reduced ability to produce ammonia in the kidneys and, in the untreated state, osteomalacia or rickets.

In addition, the three cases described presented the following symptoms: hyperactivity, diminished reflexes, and flaccid musculature with abundant subcutaneous fat. Two of the cases had marked arching of the frontal bones. In all three cases, hydrophthalmus was present and two had cataracts. In addition, misty corneae and blindness and coarse, wandering nystagmus were present. All three patients uttered now and again a strange ringing cry and, during the course of the disease, there were periods of pyrexia without demonstrable cause. As regards the laboratory investigations and other details the reader is referred to the original paper. In the following years similar cases with slight variations in the symptoms were published (3, 4, 6, 11).

During the winter of 1958–1959, two patients were admitted to the Paediatric Clinic, the University Hospital of Copenhagen in whom the clinical picture was so suggestive of that described by Lowe *et al.* that it was considered of interest to report them.

Case Histories

Case 1. Boy aged 1½ years. Only child of apparently healthy parents (see later). The maternal grandmother had cataract. The pregnancy and the delivery was normal. Birth weight 3000 g. The ocular anomaly was noticed immediately after birth and the infant was admitted to the Ophthalmic Department, the University Hospital of Copenhagen, where he was hospitalized on four occasions during the period from the age of 14 days until 16 months of age with the diagnoses of right buphthalmus and left iritis. The treatment instituted was enucleation of the right eye. (Microscopic examination of the exstirpated eye showed a greatly dilated, milky cornea and a cataractous lens. There were no abnormalities of the chamber angle, and the canal of Schlemm was normal, but there was atrophy and degeneration of the other layers, presumably secondary to glaucoma.) In the left eye iridectomy was

Fig. 1. B.C. Boy aged 1½ years with Lowe's Syndrome. Prothesis in right eye socket. Left hydrophthalmus.

undertaken. (Biopsy showed chronic iritis.) At the age of three weeks the urine was free from albumen, but at the age of 5½ months albuminuria was demonstrated for the first time (0,2 ‰) but, on the occasions mentioned, no sugar could be demonstrated in the urine. At the age of six months, pathological amounts of lysine, cystine and other amino acids were demonstrated in the urine.

At the age of 16 months the child was admitted to the Paediatric Clinic, the University Hospital of Copenhagen. The weight was then 8060 g, the length 71 cm and the circumference of the head 47 cm. The child was pale and thin and was retarded both mentally and somatically. The cranium was scaphalocephalic with a comb-shaped sagittal suture. The fontanelle could just be felt. The right eye socket contained a prothesis. The left eye, which was considerably enlarged, showed nystagmus (Fig. 1). The pupil was large but the tension was not increased. Only perception of light was retained. The hearing was greatly reduced and the child reacted only to loud noises. The musculature of the extremities was poorly developed, the tone was reduced and the reflexes weak but normal in type. Slight hypermobility of the joints was present. The child lay, as a rule, passive on his back with stereotype flapping movements in front of the eyes. Now and again he made babbling noises. The child had difficulty in retaining food. Vomiting occurred frequently and there was extreme constipation. Now and then during the course of the disease there were brief periods of pyrexia without demonstrable cause, but no characteristic cry. No cystine crystals were found in the cornea.

Laboratory Investigations: Albuminuria was demonstrated but no glucosuria. Microscopic examination of the urine was normal. The micro-ESR was consistently raised and varied between 17 and 44 mm per hour. A constant slight anaemia was observed. The urine contained cystine, lysine, histidine, alanine, and glycine in large quantities and, in addition, serine, glutamic acid, aspartic acid, glutamine, threonine, proline, valine, leucine, methionine, phenylalanine, trypto-

TABLE 1. *Laboratory investigations in Case 1.*

	On ordinary diet	On 4 % semolina gruel	On 4 % semolina gruel after 15 days with 100,000 Units vitamin D daily	On ordinary diet 15 days after withdrawal of vitamin D
Blood				
Micro ESR in mm	17	25	25	
Haemoglobin %	65	75	79	
Blood urea mg %	22		58	19
Serum chloride mEq	95			95
Serum bicarbonate mMol	16	18.5		15
Serum potassium mEq	4.6			3.7
Serum sodium mEq	138			138
Serum calcium mg %	10.6	10.4	12.6	10.9
Serum phosphorus mg %	3.4		7.3	3.7
Alkaline phosphatase units	50	38	28	16
Amino acids mg %		4.9		
Urine				
Quantity per 24 hours	400	240	465	
Specific gravity		1033	1019	
Albumen	+	+	+	+
Glucose	−	−	−	−
pH	9	9	8.9	
Sodium mEq	25	14	14	
Potassium mEq	24	24	37	
Calcium mg	60	23	26	
Phosphorus mg	288	463	330	
Chloride mEq	35	18	13	
Faeces				
Quantity per 3 days		117.1 g		
Calcium per day		1200 mg		
Phosphorus per day		367 mg		
Average consumption per day of				
Calcium		1220 mg	1220 mg	
Phosphorus		970 mg	970 mg	

phane, tyrosine and arginine, as determined by paper chromatography (undertaken by A/S Medicinsk Laboratorium, Copenhagen). EEG: Slightly abnormal curve. For the other laboratory investigations see Table 1. Radiographic examination: no definite skeletal changes. Urography: normal.

No information was available regarding the administration of vitamin D during the period prior to the age of 16 months.

As the reaction to treatment with massive doses of vitamin D was considered to be of interest, the child was placed on a constant diet of 4 % semolina gruel containing 140 mg % calcium and 112 mg % phosphorus. After 14 days this diet was supplemented with 100,000 units vitamin D daily for a total of 15 days and the blood and urine again investigated (see Table 1). Repeated vomiting still occurred and severe constipation persisted. A few weeks after the withdrawal of the massive doses of vitamin D the blood urea rose and, for this reason, copious amounts of fluid and sodium bicarbonate were administered, and the tolerance test was discontinued. Following the return of the blood urea to normal, the patient was allowed home for Christmas. He

was re-admitted a month later. He had been quite well until a week prior to admission when pyrexia of 39–40°C had developed associated with cramps in the hands. His condition gradually deteriorated and death eventually supervened during a period of hyperpyrexia.

Autopsy: Macroscopic examination of the brain: slight meningitis, severe cerebral oedema, slight encephalitis with chronic atrophy of the ganglion cells. Porencephaly of the mesencephalon. Microscopic examination of the kidneys showed no vascular or glomerular changes. The afferent convoluted tubules were normal in structure without epithelial changes and in the efferent convoluted tubules colloid-resembling deposits were found. The renal cortex was patchily infiltrated with lymphocytes and leucocytes and isolated shrunken glomeruli were encountered in such regions. In some of the collecting tubules amorphous deposits were found and in isolated places these deposits resembled calcium. Fine fluffy deposits were also localized to the interstitial tissue. No double refractive crystals were observed. The mucous membranes of the pelves were well preserved. No crystals were demonstrated in any of the organs.

The patient's mother was examined during the child's stay in the Ophthalmic Department and albuminuria with marked cystinuria was disclosed but paper chromatography, performed in view of the amino acids in the urine, gave negative results. Examination of the eyes revealed punctate opacities in both lenses.

Case 2. Boy aged $3\frac{1}{2}$ years. Only child of healthy parents. The pregnancy and delivery was normal but the infant was cyanotic at birth. Birth weight 3700 g, length 57 cm. The infant was artificially fed and thrived well. Supplements of 800 units of vitamin D and 4000 units of vitamin A daily were administered and ascorbic acid was given from the age of 1–2 months.

First admission to Hospital: The child was admitted to the Paediatric Clinic of the University Hospital of Copenhagen at the age of six months because of retarded development. The child was big and well nourished and lay relaxed in bed. The cranium was narrow, scaphocephalic, with a circumference of 45 cm. The fontanelle was of normal size and tension. The eye movements were uncoordinated. Some convergent strabismus was present and there was occasional horizontal nystagmus. Bilateral cataracts were present and the child did not appear to be able to see. He listened to the rattling of a toy but did not try to catch it. He could hold objects but was not interested in them. He was unable to lift his head while lying on his back, but lifted it well while lying prone. The child could not sit but made attempts to crawl in the prone position. Weight was 7250 g, length 66.5 cm.

Albuminuria was demonstrated but microscopic examination of the urine was normal. No glucose was found in the urine and the blood urea was normal. The serum calcium and phosphorus were normal, alkaline phosphatase was slightly raised (42 units/100 ml). Phenylpyruvic acid could not be demonstrated in the urine but on repeated examinations cystine was found in large quantities. Large quantities of alanine and glutamine were, similarly, found, and in addition, a number of other aminoacids: glycine, serine, threonine, glutamic acid, valine, leucine, proline, tyrosine, histidine and lysine as determined by paper chromatography (performed by A/S Medicinsk Laboratorium, Copenhagen). X-ray examination revealed rachitic changes in the distal epiphyses of the extremities. Cystine crystals could not be demonstrated by split-lamp examination of the cornea. The ocular tension was normal. EEG: normal.

It was thought that the usual form of cystinosis was not involved here and the condition was, therefore, interpreted as a form of rickets. The patient was treated thereafter with 8000 units vitamin A and 1600 units vitamin D daily. Cataract operations were then undertaken on both eyes and the child could, thereafter, follow light and look at his fingers. He was discharged from Hospital on the above mentioned dose

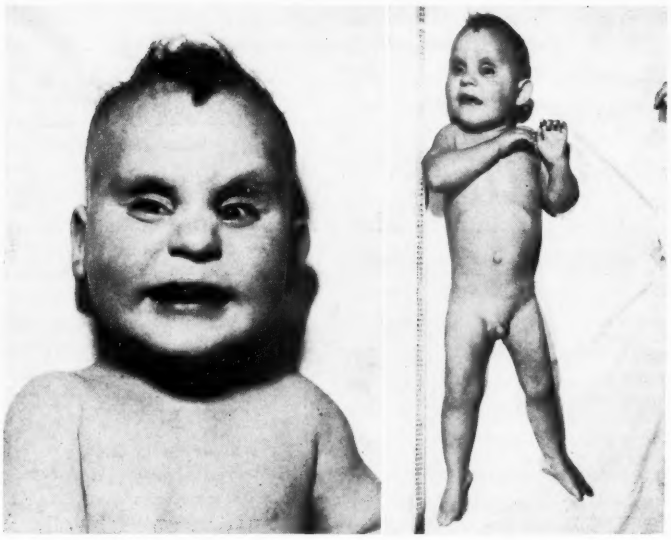

Fig. 2. J.A. Boy aged 3½ years with Lowe's syndrome.

of vitamins to a home for the care of the blind.

Contact with the child was not maintained. The child was in a childrens home and it is known, that only slight progress was made during the next three years. Nothing is known concerning the administration of vitamins during these three years.

Second admission to Hospital: The boy was readmitted to the Paediatric Clinic, the University Hospital of Copenhagen at the age of 3½ years for control of the condition. No illness had occurred apart from a tendency to catarrhal conditions. Length was 90 cm, i.e. 9 cm less than the average for the age, and the weight was 12.5 kg. The child lay flat on his back and made almost continual stereotype bending and stretching movements particularly of the upper limbs. He could sit almost without support but was unable to support himself on his legs. He could lift the head and body in the prone position. The cranium was narrow, but otherwise normal. The fontanelle was closed and the circumference of the skull was 48.8 cm. There was no evident craniotabes. The eyes were deeply set with rather large corneae, rather small pupils and definite bilateral central cataracts. Perception of light was possibly present. No definite strabismus was present. The eye movements appeared to be co-ordinated but occasionally nystagmoid movements occurred. There was a suggestion of a rickety rosary. The tone of the extremities was reduced, there was some hypermobility of the joints and the tendon reflexes were weak. The hearing appeared to be severely reduced.

Albuminuria was again demonstrated together with aminoaciduria (comprizing cystine, glucine, serine, glutamic acid, glutamine, alanine, threonine, histidine, valine, leucine, phenylalanine and lysine in large quantities), but no glucosuria was demonstrated. No definite rachitic changes were observed on x-ray examiation of the extremities, but the distal end of the ulna was, however, slightly broader than normal. Intramuscular urography: right hydronephrosis, bilateral nephrolithiasis, possibly defective excretion on the left side. EEG-curve when awake: slightly abnormal pat-

TABLE 2. *Laboratory investigations in Case 2.*

	1956	1959		
		On artificial feeding with 1200 g semolina gruel daily	Same diet but after treatment for 14 days with vitamin D (100,000 Units daily)	Same diet 12 days after withdrawal of treatment with vitamin D
Blood				
Micro ESR in mm	5	21		37
Haemoglobin %	90	85		70
Blood urea mg %	38	70	59	19
Serum chloride mEq	111	130	103	109
Serum bicarbonate mMol	20	14	19	17
Serum sodium mEq	149	145	146	147
Serum potassium mEq	4.2	4.2	4.3	3.7
pH		7.31	7.39	7.33
pCO_2 mm		32	34	35
Serum calcium mg %	9.8	9.7	10.3	11.3
Serum phosphorus mg %	4.5	4.2	5.3	6.7
Alkaline phosphatase units	42	38.8	29.0	15.0
Amino acids mg %		4.5	4.6	4.5
Amino acids chromatogram		normal	normal	normal
Urine				
Quantity per 24 hours ml		620	720	540
Specific gravity		1011	1012	1012
Albumen	+	+	+	+
Glucose	–	–	–	–
pH		6.5		
Sodium mEq		40	14	14
Potassium mEq		31	43	30
Calcium mg		176	92	15
Phosphorus mg		409	864	535
Chloride mEq		35	33	9
Ammonium g		0.5	0.3	0.3
Amino acid chromatogram	pathological	pathological	normal	pathological
Netto acids mEq		20	24	17
Faeces				
Quantity per 3 days		110 g	104 g	128 g
Calcium per day		1687 mg	1075 mg	1707 mg
Phosphorus per day		880 mg	381 mg	683 mg
Average consumption per day of				
Calcium		1680 mg	1680 mg	1680 mg
Phosphorus		1200 mg	1200 mg	1200 mg
Balance				
Calcium		– 183 mg	+ 513 mg	– 42 mg
Phosphorus		– 89 mg	– 45 mg	– 18 mg

tern. Sleeping curve: moderately abnormal pattern. Fractional serum protein determination: the values for alpha$_1$-, alpha$_2$-, beta$_1$- and beta$_2$-globulins were raised, a condition which is observed in cases of nephrotic renal changes. The results of the other laboratory investigations appear in Table 2.

On the supposition that a case of the syndrome described by Lowe *et al.* was involved, a decision was made to perform tolerance tests with massive doses of vitamin D and the results of this experiment appear in Table 2. During the entire experimental period the child received a constant diet of semolina gruel containing 140 mg % calcium and 100 mg % phosphorus. A total of 1.8 million units of vitamin D was administered over a period of 18 days.

Following the favourable effect of the vitamin D therapy on the aminoaciduria and on the calcium-phosphorus balance, treatment of the child as an out-patient with large dosis of vitamin D was begun. The size of the dose was regulated by serum calcium determinations and the optimal dose appeared thus to be approximately 20,000 units of vitamin D daily. In addition sodium bicarbonate was administered in a dose of 1 g daily.

The ophthalmological changes in the two cases recorded here have previously been reviewed in detail by Seedorff.

Discussion

These two children resembled one another greatly, considerably more than the photographs, Figs. 1 and 2, suggest. When left alone both of them lay relaxed on their backs with rolling movements of the head, coarse wandering nystagmus and, singular, almost athetoid movements of the upper limbs in which they waved their hands in front of the eyes.

The author is of the opinion that both of these cases may be interpreted as cases of the syndrome described by Lowe *et al.*, viz. one of the clinical pictures resembling the de Toni–Debré–Fanconi syndrome associated with ocular changes and mental retardation. Thus, in these two patients the following were encountered: Mental and somatic retardation, hydrophthalmus (Case 1), cataract (Case 2), reduction of vision to blindness with coarse wandering nystagmus, hyperactivity, hypotonia and diminished reflexes in the extremities, hypermobility of the joints and periodic pyrexia without demonstrable cause. On tolerance tests with massive doses of vitamin D, increased phosphorus absorption from the intestine was demonstrated with an increase in the serum phosphorus values despite the increase of the already great excretion of phosphorus via the kidneys. In Case 2 radiological evidence of rickets was found while in Case 1 the defective growth was possibly the reason that the rickets had not developed. The blood alkaline phosphatase was raised in both of the patients. In addition, the following were found on examination of the blood: Acidosis, low normal to reduced serum phosphorus, normal blood urea and amino acid content. Greatly increased quantities of amino acids were demonstrated in the urine (including cystine also). In Case 1 the urine was alakaline and the specific gravity normal as in Lowe's patients while, in Case 2 the finding of acid urine and isostenuria was possibly due to the fact that in this patient more severe renal changes were present (right hydronephrosis, bilateral nephrolithiasis and possible absence of excretion on the left side). Case 1 showed constant albuminuria with normal specific gravity and diuresis and, at autopsy, only slight evidence of subacute-chronic pyelonephritis was found.

This slight urinary condition cannot explain the acidosis, hyperphosphaturia, or the aminoaciduria On the other hand, Case 2 had real renal disease with radiographic changes and changes in the urine: isosthenuria, albuminuria, hyperphosphaturia and acidosis in the blood. The sodium, calcium and chloride values in the blood, were, however, found to be normal while these values are frequently reduced in chronic renal diseases. The serum phosphorus was low normal while in chronic renal conditions this is frequently raised. Further, the aminoaciduria in this case was considerably greater than that seen in renal diseases and this observation, together with the presence of other defects is evidence that, at least, some of the symptoms may be explained as the result of inborn errors of metabolism.

In neither of the two cases could cystine deposition be demonstrated so that the diagnosis of Lignac-Fanconi's syndrome may be excluded. On no occasion was steatorrhoea or any other abnormality of alimentary excretion observed which might explain possible poor resorption of vitamin D.

In Case 2 the rachitic changes diminished or perhaps entirely disappeared on approximately 1600 units vitamin D daily (information very unreliable). The reason for this is somewhat obscure. Perhaps the fact that the child's growth in the intervening period was considerably inhibited (weight: 7850 g→12.500 g, length: 73→90 cm) played a part so that despite a greater requirement of vitamin D than normal, the child could manage on an inadequate supply of the vitamin. It should be noted that the centres of ossification in this case correspond to the age.

In patients with de Toni–Debré–Fanconi's syndrome, large doses of vitamin D have a favourable modifying effect on the hypophosphataemia, the high renal phosphate clearance, glucosuria, aminoaciduria and the hyperchloraemic acidosis (Salassa, Power, Ulrich & Hayles). Trial administration of large doses of vitamin D to the two patients described here had a correspondingly favourable effect as the serum calcium and phosphorus balances improved. The acidosis became less pronounced and the excretion of amino acids in the urine became completely normal. On the other hand, the author does not consider it justified to draw any conclusions from the more uncertain changes in the potassium, sodium and chloride values in the blood and the urine.

It is a peculiar fact that, during treatment with large doses of vitamin D not only does an increase occur in the serum phosphorus values but also in the excretion of phosphorus in the urine. This observation does not fit in with the theory propounded by Albright *et al.* (2) according to which the effect of vitamin D consists of increased calcium absorption→increase in serum calcium→reduced parathyroid activity→reduced excretion of phosphorus in the urine and, finally, increase in the serum phosphorus (unless it may be presumed that the parathyroid function is greatly reduced; a fact which the serum calcium values do not suggest). Nor does the observation agree with Dent's theory according to which the effect of vitamin D consists of a direct effect upon the renal tubules so that reabsorption of phosphorus is increased. In normal individuals the excretion of phosphorus is increased with acidosis but this cannot ex-

plain the increase either since during the treatment with vitamin D a reduction of the acidois occurred. Vitamin D causes an increase in the absorption of phosphorus from the intestine (2, 5, 12) and in its resorption in the renal tubules (5, 7, 12). As in these two patients, the author found an increase in the excretion of phosphorus in the urine simultaneously with an increase of the values for the phosphorus in the blood associated with a reduction of the faecal content of phosphorus (this latter was, unfortunately, only demonstrated in Case 2), it seems as if the increase in absorption of phosphorus from the intestine must have been the most important effect of vitamin D on the phosphorus metabolism. Possibly the lesion in the renal tubules involving the resorption of phosphorus in these two cases was not so susceptible to vitamin D but the phosphorus balance, nevertheless, improved during treatment with vitamin D.

It is interesting that with the high phosphate clearance no simultaneous glucosuria was found but glucose and phosphate do not necessarily need to correspond; cf. conditions in renal glucosuria, where only glucose is excreted and, similarly, aminoaciduria may occur without simultaneous glucose or phosphorus excretion, e.g. in certain cases of hepatolenticular degeneration and in cystinuria (Dent).

The serum amino acid content was normal so that the aminoaciduria must be of renal origin (cf. Dent).

The hypotonia in the two cases can scarcely be explained by the hypopotassaemia as this, in any case, was not demonstrated with certainty.

As regards treatment it may be remarked that while the three patients mentioned by Lowe *et al.* required only moderate doses of vitamin D to improve, both of the patients described here required considerably raised doses of vitamin D in order to attain approximately normal blood and urine values.

Both of the patients described here were very deaf and EEG changes were demonstrated in both of them. In Case 2 the EEG had previously been normal. In Case 1 definite histological changes in the brain were found at autopsy.

Finally, it should be mentioned that in Case 1 hereditary conditions of interest could be demonstrated as the patient's mother had albuminuria, cystinuria and opacities in both lenses.

Summary

Two cases of Lowe's syndrome are recorded. By means of investigations with the administration of large doses of vitamin D, increased phosphorus absorption from the intestine with an increase in the serum phosphorus values despite increase in the already high excretion of phosphorus in the urine were demonstrated. Doses of approximately 20,000 units of vitamin D were necessary therapeutically and it appears that this dosage must be maintained.

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CASE REPORT

Galactosaemia

Report of a Case[1]

by OLE MORTENSEN and GUNNAR SØNDERGAARD

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We have previously reviewed galactosaemia (15) and reported a case (5, 16). Since then, many additional cases have been described in the literature. Among reviews published during recent years we may mention those by Schreier *et al.* (21), Schultze-Jena & Schaper (22) and Holzel *et al.* (10). Kalckar (12) suggested that the term "hereditary galactosaemia" should be used for the disease, since it has now been shown that it is attributable to some genetic factor.

Below, we present a further case, in which special attention has been given to laboratory examinations in relation to galactose-tolerance tests.

Case Report

A boy, aged 3 months, was admitted to the Paediatric Department on Nov. 22, 1954. He was the youngest of three siblings; the two others and the parents were in good health. There were no known instances of infant deaths, cataract or mental deficiency in the family. The pregnancy had been normal, apart from vaginal bleedings in the first or second month of pregnancy. The boy had been delivered by Caesarean section. He was breast-fed for one week and afterwards given a milk formula with added milk powder. Neonatal jaundice was present for 3 weeks, and the boy was strikingly quiet and slept much. He lost 700 g in weight during the first fortnight.

At the age of 2 months, the patient was admitted to a regional hospital with violent diarrhoea. He was critically ill, dull and greyish pale. On admission, the body weight was 3500 g. He was treated with blood transfusions, Darrow's solution, streptomycin, penicillin, etc. The urine was not examined for sugar. As considerable enlargement of the liver was revealed, he was transferred to this department at the age of 3 months.

On admission, the patient was emaciated, pale and flappy. Weight 3950 g, length 55 cm. He could smile and fix his attention on objects. The abdomen was large and tense. The liver extended to the right iliac crest and to the middle of the left costal margin. The spleen was palpable 3 cm below the costal margin. Ophthalmological examination revealed greyish diffuse blurring of the central portions of both lenses and some blurring more peripherally. Neurological examination did not reveal any abnormalities.

[1] Aided by a grant from Nordisk Insulinfond o G.S.

Protein and galactose were present in the urine. As a diagnosis of galactosaemia was thus unquestionable, the patient was placed on a milk-free diet, following which sugar and protein disappeared from the urine. The boy began to gain weight and thrived satisfactorily. The size of the liver decreased, and 4 months later the cataract had disappeared.

At discharge, the boy, now being 8½ months old, weighed 7850 g; length 66 cm. He was alert, could sit up alone and play with toys; he was interested in his surroundings, but did show some retardation both in mental and motor development. The liver extended 2–3 cm below the right costal margin, while the spleen was impalpable.

At a follow-up examination at the age of 4 years, the somatic condition was satisfactory, but some mental retardation was noted.

Fig. 1. One-dimensional sugar chromatogram of urine of the patient while on a milk diet. 1, urine; 2, urine + glucose; 3, glucose + galactose; 4, urine + galactose.

Laboratory Examinations

Methods

The urinary excretion of amino acids was studied by two-dimensional paper chromatography on Whatman paper No. 1, with phenol-water (3 : 1, w/v) and pyridine-butanol-water (1 : 1 : 1, v/v) as the solvent. Ninhydrin dissolved in acetone was used for spraying.

The presence of urinary carbohydrates was demonstrated by one-dimensional paper chromatography with butanol-pyridine-water (10 : 5 : 7, v/v) as the solvent. The spray was benzidine-acetic acid, except for the demonstration of ketoses, for which naphthoresorcine-trichloracetic acid was used.

Galactose and glucose in the blood and urine were determined quantitatively by means of a previously described micromethod using glucose oxidase and anthrone (SØNDERGAARD (26)).

Results

As already mentioned, sugar was present in the urine during the first few days in Hospital while the infant was still on an ordinary milk formula. By determination of the melting point of osazone (performed by S. Darling, Ph.D.) and paper chromatography (Fig. 1), this sugar was identified as galactose. No other carbohydrates could be demonstrated in the urine.

The fasting blood-sugar level (determined by the method of Hagedorn and Jensen) was 110 mg % and, 3 hours after ingestion of milk, 200 mg %. When the infant was on a milk-free diet, glucose-tolerance tests showed normal values.

When the boy had been on a galactose-free diet for 5 months, a galactose-tolerance test with 12.6 g galactose (1.75 g/kg body weight) was performed. Eight hours later, the body temperature had risen to 39°C, and the patient vomited once. On the next morning, the temperature had returned to normal, and no other untoward reactions were seen.

The galactose-tolerance curve (Fig. 2 was greatly abnormal with a maximun concentration of 378 mg % at 90 minutes; at 2½ hours the galactose concentration wa

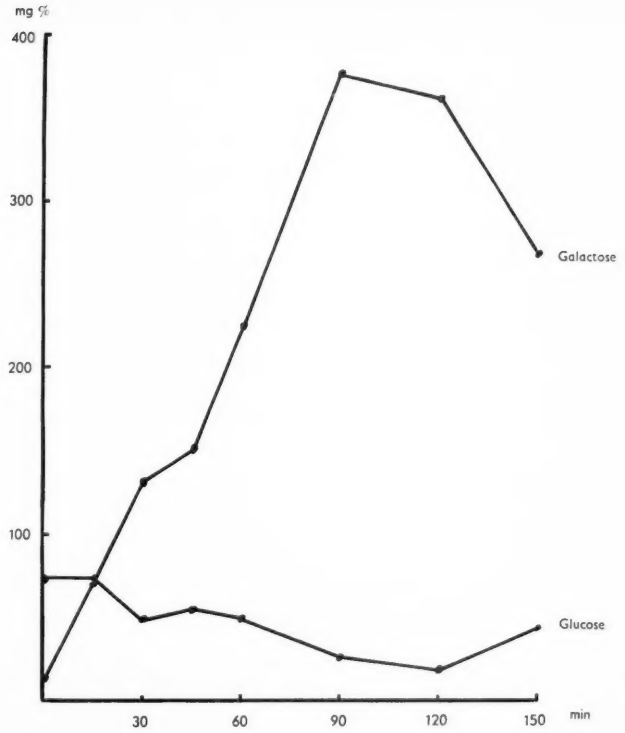

Fig. 2. Galactose-tolerance test. Upper curve: galactose; lower curve: glucose.

still 266 mg %. During the test, the blood concentration of glucose showed a considerable fall, from 75 mg % to 18 mg % at 120 minutes, but no hypoglycaemic reactions were observed.

All urine samples collected during the first 24 hours after the administration of galactose were examined for sugar. The amounts of glucose and non-glucose were determined, and at the same time the concentrations of the individual carbohydrates were estimated by means of paper chromatography on the basis of the size and intensity of the spots. A similar quantitative estimation of the urinary excretion of amino acids was done by one-dimensional paper chromatography. The concentration of inorganic phosphate was determined in some of the urine samples. The results of these examinations are shown in Table 1. About one half of the ingested galactose was recovered from the urine. In addition, 2.1 g glucose was excreted.

As might be expected, paper chromatography of the individual urine samples (Fig. 3) showed a considerable excretion of galactose, which ceased within 25 hours. In agreement with the quantitative determinations, distinct excretion of glucose occurred at the same time; it began a few hours after the administration of galactose and reached a peak at 6 hours, i.e., a little later than the maximum excretion of galactose. At 24 hours, glucose could no longer be demonstrated.

Simultaneously with the decrease in the

TABLE 1. *Urinary excretion of amino acids, carbohydrates and inorganic phosphate after oral administration of galactose.*

	Hours after administration of galactose	Urinary output ml	Amino-aciduria	Glucose %	»Galactose» %	Fructose	Phosphate mg %
1	0^{45}	34		0	0.55	0	11.7
2	2^{50}	35		0.14	5.35	0	10.4
3	3^{30}	20	0	0.14	5.45	0	
4	5	49	(+)	2.59	3.60	(+)	18.3
5	6^{30}	31	(+)	2.07	3.08	(+)	29.3
6	8^{30}	9	+	0.67	1.15	+	34.4
7	11^{30}	5	+ + +	1.78	1.48	+ +	
8	22	29	+ + +	0.20	0.54	+	66.0
9	23	8	+ + +	0.02	0.21	(+)	
10	24^{45}	25	+ +	0.10	0.04	0	28.3

excretion of galactose, another carbohydrate with $R_{Gluc.}$ 1.24 was observed. The spot stained reddish yellow with naphthoresorcine, and an aqueous extract of the spot gave a positive Seliwanoff reaction. It is therefore likely that the carbohydrate was fructose. In addition, a very weak spot of the same position as xylose was observed. Finally, another very weak spot with $R_{Gluc.}$ 0.56 was seen; it became more distinct and reddish yellow on spraying with naphthoresorcine-trichloracetic acid. This substance was thus presumably saccharose.

One-dimensional chromatography (Fig. 4) of the individual urine samples showed considerable excretion of amino acids. It began slightly a few hours after the administration of galactose; it was most vigorous at 11–23 hours and was still distinct at 25 hours.

Two-dimensional paper chromatography performed 22 hours after the administration

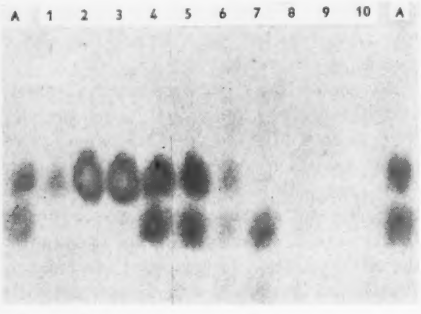

Fig. 3. Carbohydrate chromatogram of urine during the first 25 hours after oral administration of galactose. A, galactose + glucose; for 1–10, see Table 1.

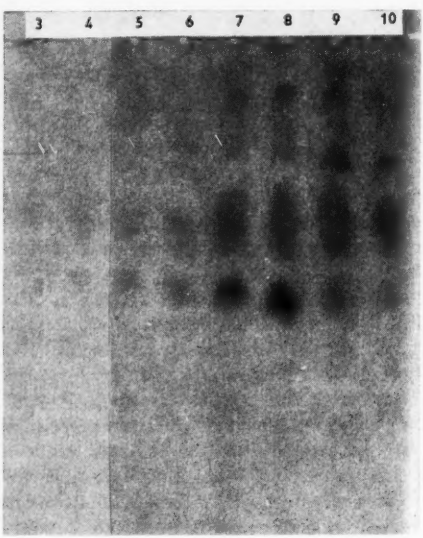

Fig. 4. Amino-acid chromatogram of the urine during the first 25 hours after oral administration of galactose. For 3–10, see Table 1.

of galactose revealed the following amino acids: alanine, valine, leucine, glutamic acid, serine, glycine, glutamine, citrulline (?), histidine, tyrosine, aspartic acid, threonine, proline, α-aminobutyric acid and cysteine.

The excretion of inorganic phosphate increased considerably during the first 24 hours (Table 1).

As previously mentioned, the blood concentration of glucose fell during the galactose test to 18 mg % without concurrent hypoglycaemic reactions. In order to study if the infant responded to hypoglycaemia when the blood did not contain galactose, an intramuscular injection of 9 I.U. of insulin was given. In a little less than 1 hour, at a time when the blood-sugar level was 39 mg %, signs of hypoglycaemia were observed, viz. irritability, pallor and motor agitation followed by clouding, and later complete loss, of consciousness with clonic convulsions. The blood-sugar level was then 18 mg %. These reactions subsided as soon as glucose was administered through a stomach tube.

Discussion

The case history reported above is characteristic of congenital galactosaemia. The diagnosis could be made on the basis of the clinical symptoms and the demonstration of galactose in the urine, so that a galactose-tolerance test was unnecessary in this case.

It is sometimes stated in the literature that galactose-tolerance tests should be omitted in order to avoid that an infant with galactosaemia is exposed to a toxic influence. However, although methods have now been elaborated by which the diagnosis can be secured without the use of galactose-tolerance tests—viz. by the demonstration of an accumulation of galactose-1-phosphate in the red cells (23) or by a specific enzymatic assay (1)—these methods are too complicated for clinical routine work. As long as simpler methods are not available, a galactose-tolerance test will presumably be required in cases of doubt. Administration of galactose on a single occasion will scarcely do much additional harm in an infant who has for some time been on a milk diet. On the other hand, a newborn or greatly debilitated infant should obviously not be exposed to any needless risk.

The toxic effect of ingestion of galactose was seen distinctly in our patient, who responded with fever and vomiting to the galactose test which was performed after the infant had been on a milk-free diet for 5 months at a time when the general condition was good.

Fever of unknown cause has repeatedly been reported in patients with galactosaemia. Goldbloom & Brickman (8) assumed that the cause might be lowered resistance to infections due to a low serum-globulin level. The rise in temperature which occurred a few hours after the administration of galactose in our patient shows that this reaction may somehow be referable to a more direct effect of galactose.

In addition to galactosuria, the chromatographic studies revealed excretion of considerable amounts of glucose. That this is not an accidental finding appears from the fact that urinary excretion of glucose was also observed in another patient with this disease (Darling & Mortensen (5)). Other authors (3, 19, 25) have also observed glucosuria in patients with galactosaemia. It is therefore scarcely justified when Bickel (2) claims that urinary excretion of carbohydrates other than galactose is suggestive of hepatic

disease, whereas only galactose is excreted in galactosuria.

Both the glucosuria, amino-aciduria and the increased urinary excretion of phosphate after the ingestion of galactose are suggestive of an impairment of the function of the proximal renal tubules, reminiscent of the conditions observed in the de Toni-Debré-Fanconi disease. The albuminuria which is seen in nearly all cases of galactosaemia may possibly also be due to a defective reabsorption of protein in the renal tubules (Rather (20)).

In a case of galactosaemia, Butler & Flynn (3) observed that the protein excreted mainly consisted of α_2-globulin, and made the same observation in other diseases associated with renal tubular disorders. While Fanconi's disease is probably due to a primary enzymatic defect in the tubules, a secondary toxic effect of galactose is exerted in galactosaemia, since the pathological components disappear as soon as galactose is eliminated from the diet. On the other hand, it can scarcely be doubted that both the glucosuria and the amino-aciduria are of renal origin, since the blood concentrations of glucose and amino acids are not increased unless severe liver damage is present, which is rarely encountered in such patients.

The mechanism of the toxic action of galactose on the renal tubules has not yet been clarified. It is reasonable to assume that galactose to a greater or smaller extent blocks the reabsorption of the other substances. This might, for example, be the case if the tubules contain a factor which is utilised in the reabsorption of several different components of the glomerular filtrate. However, most investigators who have concerned themselves with this problem (10, 21) express the view that a competition between galactose and amino acids is not a plausible explanation of the amino-aciduria, because this condition does not occur until the patient has been on a galactose-containing diet for some days, and it does not disappear again until several days after the elimination of galactose from the diet.

The observation that glucosuria and amino-aciduria occurred in our patient a few hours after the administration of galactose and decreased markedly within 24 hours is in favour of the assumption that galactosuria may nevertheless exert a competitive action on the reabsorption of amino acids and glucose.

The explanation might be a competition for transport through the cell membrane, in analogy with the penetration of hexoses into erythrocytes and muscle cells (Park *et al.* 18). Intracellular disturbances in intermediate metabolism referable to an abnormally high concentration of galactose or galactose-1-phosphate may also be a decisive factor. Sidbury (24) observed that galactose-1-phosphate, in erythrocytes in vitro from patients with galactosaemia, competitively inhibits carbohydrate metabolism, and Penington & Prankerd (19) found that the concentration of erythrocytic adenosine triphosphate decreases in patients with galactosaemia on a milk diet.

Some of the other carbohydrates which were demonstrated in the urine after the administration of galactose may possibly have originated from the diet, since the patient at the time when these substances were excreted in the urine had been given his usual milk-free feeding. Although saccharose is normally not absorbed from the intestine, this may occur in certain

pathological conditions, such as 'idiopathic saccharosuria' (14). The excretion of the carbohydrates ceased simultaneously with the galactose excretion, so that galactose must be assumed to be responsible for the urinary elimination of carbohydrates.

At the present time it is not possible to say anything decisive as to the cause of the amino-aciduria and melituria; various factors may have been contributory. Date (6) thus observed melituria in febrile conditions, and Choremis *et al.* (4) revealed amino-aciduria in thirst fever.

During the galactose-tolerance test, the blood concentration of "true" glucose fell to 18 mg % without concurrent hypoglycaemic reactions. On the other hand, after injection of insulin hypoglycaemic reactions occurred already at 38 mg % (determined by the anthrone method). This value is only a few mg % above the blood level of glucose, since the residual sugar in this patient had repeatedly been determined as 5–6 mg %. Thus, it cannot be doubted that symptoms of hypoglycaemia after the insulin injection developed at a glucose concentration which was higher than that we observed during the galactose-tolerance test without accompanying hypoglycaemic symptoms.

Hypoglycaemia during galactose-tolerance tests in patients with galactosaemia has repeatedly been mentioned in the literature, sometimes even with very low glucose values without hypoglycaemic reactions. Even though we made the same observation in our previous patient (16), we did at that time express scepticism as to the exceptionally low blood-glucose levels, because the usual technique with removal of glucose by means of yeast involves certain sources of error.

On the basis of animal experiments it must be expected that galactose is capable of counteracting hypoglycaemic reactions only to the extent it is converted into glucose. Thus, Noble & Macleod (17) observed hypoglycaemic convulsions in rats in spite of a high blood concentration of galactose, and Maddock *et al.* (13) found that galactose was unable to abolish the hypoglycaemic changes in the EEG from hepatectomised animals. Following an insulin injection into a child with galactosaemia, Greenman (9) observed hypoglycaemic reactions in spite of an increased level of total blood sugar.

In order to avoid the sources of error involved in the use of yeast, we used glucose oxidase in this patient and found, as previously mentioned, considerable hypoglycaemia unassociated with clinical manifestations. The same phenomenon has been observed by other authors. On the other hand, Townsend *et al.* (27) and Holzel *et al.* (10) observed hypoglycaemic convulsions after administration of galactose in children with galactosaemia. The problem as to whether galactose in some patients with this disease may afford protection against the development of hypoglycaemic symptoms is as yet unsolved. The cause of the hypoglycaemia is also unknown, but Isselbacher (11) suggests that it may be referable to increased insulin secretion from the pancreas due to a high blood level of galactose. Dormandy *et al.* (7) found that intravenous injection of galactose resulted in hypoglycaemia in normal subjects.

Summary

A case of galactosaemia in a 3-month-old boy is reported. A galactose-tolerance

test performed after the patient had been on a milk-free diet for 5 months showed a markedly pathological result, and pronounced hypoglycaemia without corresponding clinical symptoms was observed. Glucose oxidase and anthrone were used for the blood-sugar analysis. A few hours after the administration of galactose, paper chromatography showed considerable amino-aciduria and excretion of galactose, glucose and a few other carbohydrates; the excretion of phosphate likewise increased.

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Swedish Pediatric Society

Meeting February 12, 1960

C. G. Bergstrand and O. Qvist: **Late prognosis of operatively treated bilateral cryptorchism**

During the 12-year period from 1934 through 1945 fortyfour boys with bilateral cryptorchism were operated at Kronprinsessan Lovisas Barnsjukhus. At a follow-up study 1959 it was possible to trace all patients but 3. Two patients were excluded as they were still too young. The remaining 39 patients (operated upon at an age of 3 to 14 years) were divided into four groups: fertile, possibly fertile, sterile and probably sterile patients according to certain criteria. These were paternity, testicular findings and if available sperm-counts. It is concluded that in this material between 35 and 55 per cent of the patients were fertile. The study also suggests that a correct anatomical result of the operation is of great importance for future fertility.

Discussion. *B. Vahlquist:* There does seem to be a connection between the operative result and fertility. Is there any connection between the original position of the testicle and the operative result? — *Bergstrand:* The question is not quite so simple to answer from the foregoing material. In general it seems likely that a more favourable "initial position" will give a better operative result than will be the case if the testicle lies, for example, in the abdomen. A primary satisfactory operative result, ought to indicate future fertility, but does not guarantee it. — *R. Lundström:* Since a significant number of cases of cryptorchism undergo spontaneous descent of the testicle, any consideration of the prognosis in early as opposed to late operation, must take account of the fact that with early operation one would operate on cases that would have undergone spontaneous correction. A priori one ought therefore to expect a better prognosis in those operated early, as opposed to, late, without allowing for the operation itself. — *Bergstrand:* That is certainly correct, but the difficulty lies in deciding in which cases spontaneous descent will ensue. Neither is spontaneous descent a guarantee of fertility. In this respect, the time of descent of the testicle probably plays a very important role. — *K. Kaijser:* Can one from the above material draw any conclusions about the most suitable age for operation in a boy with bilateral cryptorchism, in order that he may have the best prospects for fertility as an adult? — *Bergstrand:* No, the material is too small to allow definite conclusions on this point. There is, however, no tendency for a poorer result in those cases where operation was carried out relatively late.

B. Blomquist, R. Lundström and C. Thorén: **Passive Immunization Against Rubeola in Pregnancy. A Follow-Up Study.**

In the course of a rubeola epidemic in Sweden in 1950–51, encouraging results were obtained with the use of convalescent serum. With a new epidemic of rubeola in 1955–56, pregnant women who had never had the disease, and had been exposed to it in any of the first four months of pregnancy

were given 4 ml of immune globulin at the Crown Princess Louise Children's Hospital. Seven cases (2,8 %) of rubeola occurred among 252 women thus treated, all of whom received treatment within a week of exposure. Follow-up study of the children of these women was performed between 2 and 4 years of age. Hearing defect and chorioretinitis was found in one of the children of the mothers who developed the disease despite prophylactic treatment. Among the 245 women who did not develop the disease after prophylaxis, there was also one case with malformations of the rubeola type, namely showing cataract and a congenital heart lesion verified at post-mortem examination. Furthermore, there was in this series one small polar cataract, as well as one case with a mild ventricular septal defect of the Roger type. During the course of the epidemic the available supply of immune globulin (SBL) was exhausted. Twenty seven women therefore received ordinary gamma globulin (KABI). In this group there were no cases of rubeola nor of rubeola damage in the children. Thirty women who became ill with manifest rubeola were treated with 12 ml of immune globulin. In four of the children of this group, follow up study revealed damage of the rubeola type. The follow up examination is as good as complete, as only one of the children in this series has eluded medical examination. It appears reasonable to suppose that immune globulin, in the employed dosages, as well as gamma globulin, prevented the appearance of manifest rubeola to a certain extent. The occurrence of typical and grave stigmata of the rubeola type in one of the children would indicate that the mother's illness in this case ran a subclinical course. The experience with this group has motivated an increase in the dose of immune globulin in rubeola prophylaxis to 12 ml. On the other hand, in this series, 12 ml of immune globulin given to women with manifest rubeola did not obviously reduce the expected frequency of rubeola embryopathy.

Discussion. *B. Vahlquist:* Two questions. How late in the incubation period can one give immune globulin and still expect a good result? What are the results of investigations in other countries.? — *R. Lunström:* The incubation period of rubeola has been shown to have individual variations, and there does not exist any experimental work which has defined the time periods in question as has been done for measles. The recommendations we follow are: Immune globulin should be given as soon as possible after the exposure and at most no later than 8 days following it. If rubeola occurs when the immune globulin has been given later, it should thus not be considered as a failure. Experiences from other countries are not easy to interpret, likely as a result of the varying dosages and qualities of the immune globulin used.

S. Söderlund: Some Views on the Differential Diagnosis of Moist Navels

The commonest causes of a moist navel are infections and anomalies. Infections of a severe type are now uncommon. Milder infections occur with granuloma formation. In deep, whorled, navels dermatitis not uncommonly occurs. The anomalies which can result in a moist navel are patent ductus omphalo-entericus and patent urachus. At the surgical clinic of Kronprinsessan Lovisas Barnsjukhus we have, in the 17 year period from 1943–1959, treated 33 cases of moist navels. The cause in 24 cases was an omphalo-enteric duct or a patent urachus. In 7 cases the omphalo-enteric duct remained in its entirety and was open at both the navel and the small intestine (fistula omphalo-enterica totalis). In 4 cases the omphaloenteric duct was open only at the navel and ended blindly extraperitoneally (fistula omphalo-enterica partialis). A patent urachus was found in 13 cases. In two of these cases the passage was open both at the bladder and at the navel (complete urachal fistula). In the other 11 cases the patency was present only at the navel (partial urachal fistula). In one case the patient bled from a persistent umbilical artery. As a question of differential diagnosis, severe infections and dermatitis pose no problem. In the other cases it

may be difficult to determine if a fistula exists. The opening of the fistula may be difficult to discover, and part of the duct can have prolapsed and be mistaken for a granuloma. Of the 24 cases with a fistula, 6 were interpreted at the beginning as granulomas and cauterized. In the case of both omphalo-enteric fistula and urachal fistula the navel secretion is usually discovered in the first weeks of life. To determine whether the fistula originates from an omphalo-enteric duct or a patent urachus, the type of secretion is important. Intestinal contents speak for a total omphalo-enteric fistula. On the other hand, with thin, liquid, clear or purulent secretion, the fistula can originate either from the omphalo-enteric duct, or from the urachus. Fistulography is a useful diagnostic adjunct, but if the passage is small it is not always possible to force the contrast medium through it. If a patent urachus is the case, the diagnosis can sometimes be established by cystography. Probing is to be avoided, as there is a risk of perforation, and as probing does not make any noteworthy contribution to the diagnosis. A partial omphalo-enteric fistula can become entirely prolapsed. This will result in a pedunculated tumor without a fistula. Without microscopic examination it is usually not possible to decide whether a red, glistening, moist tumor in the navel is a true granuloma or represents the remains of an omphalo-enteric duct or a urachus. True umbilical granulomas are probably rarer than has hitherto been supposed. Of the 33 cases with moist navels we were unable, in 8 cases largely because microscopic examination was lacking, to decide whether infection or an anomaly was responsible. In the case of a fistula, the therapy is surgical with excision of the fistula. With a partial omphalo-enteric fistula laparotomy should be carried out, as a Meckel's diverticulum may be present. This was the case in two patients with such a fistula where laparotomy was performed. Complete omphalo-enteric fistula must be operated upon as soon as is possible, as serious complications otherwise can occur. In our material there was one case of prolapse of the intestine, and another of beginning intestinal obstruction because of a kinking of the intestine. In the case of a urachal fistula or a partial omphalo-enteric fistula operation can be postponed until the child is older. A risk of infection is present with long delay. In this series of 33 cases there were no deaths.

Åke Lundberg: Smoking Habits in a Girl's School

Sixty-six school-girls between the ages of 13 and 15 years, as well as 75 girls aged 17–20, were interviewed with regard to their smoking habits. The answers were not anonymous as the smoking habits were correlated with certain facts in the health and report cards. Three per cent of the younger girls smoked daily, 70 % never. Of the older girls 24 % were habitual smokers while 25 % denied smoking at all. The frequency information correlates well with other investigations, both within the country and from foreign sources. The parents' smoking habits probably exerted the most influence on those children who were occasional smokers. While the non-smokers and the habitual smokers were largely influenced by the habits of their friends. Daily cigarette consumption was moderate: from 1–5 a day. This may be a favourable prognostic sign, if prophylaxis were to be directed towards the younger individuals where the habit has not been firmly established. Eighty-nine per cent of the habitual smokers inhaled. Occasional smokers as well as habitual smokers showed, on the average, 10–15 more hours of absence per school year. The reports for school work and for gymnastics were not different for the smokers and the non-smokers. Precordial pains were three times as common in those that smoked. The connection can probably be found in the neuroasthenic constitution which favours the aggravation of these symptoms, as well as the increased tendency for tobacco smoking. In the older group there were 20 girls who weighed 5 kg or more over the average weight, and 14 girls who weighed 5 kg, or more less than the average. The underweight ones

were twice as commonly non-smokers compared with the overwight and normal weight girls. An asthenic constitution probably plays an important role for the explanation of this fact. The occurrence of head trauma as well as headaches without a previous history of concussion, was found oftener among those girls who smoked.

K. M. Herrlin, B. Karlsson and G. Sterky: **The EEG in Juvenile Diabetes Mellitus of Long Duration**

Of 80 patients with juvenile diabetes commencing prior to 16 years of age and of at least 15 years duration, 32 (40 %) had a normal EEG and 25 (31 %) had a pathological EEG with focal abnormalities in 12 cases, episodic dysrhythmia in 8 cases, general continuous dysrhythmia in 4 cases, and paroxysmal epileptogenic activity in 1 case. In 23 cases (29 %), the EEG was classified as borderline. The EEG was recorded at rest, under hyperventilation, and on photostimulation with a stroboscope. The EEG findings were correlated with sex, age at onset, duration of illness, patient's age at examination, occurrence of insulin or diabetic comas, occurrence of angiopathy, cholesterol level in the blood as well as the blood sugar level during the examination. These showed only that the occurrence of a pathological EEG was commoner in women than in men (40 % as opposed to 22 %). No correlation with the other factors was found. It is notable that the duration of the disease could not be correlated with the occurrence of a pathological EEG.

Finnish pediatric association

Meeting February 13, 1960

R. S. Illingworth (Sheffield, Great Britain): **The Difficulties of Developmental Prediction**

Disagreement was expressed with those who have said that developmental tests in infancy are of no predictive value. The reasons for disagreement were given, and figures were shown, indicating that mental retardation can be diagnosed with a high degree of accuracy in the first year, excluding mongols, cretins and hydrocephalics.

Developmental prediction must be based on the history, physical and developmental examination, and on the interpretation of one's findings. It must never be based on clinical impression. One must be sure that one has elicited the baby's maximum performance in each field of development: that due allowance has been made for prematurity, any illness, error of management, physical or sensory defect which may have affected his development: and one must know the relative significance and importance of different fields of development. Gross motor development is the least valuable field for predictive purposes: some mentally defective children are able to sit and walk at the usual age. Of far more importance is the child's interest, alertness, ability to imitate, concentration, vocalisations and later speech; manipulative development and chewing are also useful fields for study.

One must always remember that delayed maturation may occur, so that a child appears to be seriously backward in the early months but proves to be normal or nearly normal later: this delayed maturation is liable to occur in cerebral palsy. There may be deceptive periods in which the child apparently stops developing and retardation in single fields of development is common.

What we attempt to do is to assess the child's developmental potential; but we cannot predict what illnesses he will have in the future and which will affect his progress

we cannot predict the environmental factors at home and at school which will affect his performance. We cannot predict his personality which will affect his future.

It was emphasised that success and even genius is certainly not just a matter of the intelligence quotient: there are many other factors involved. In any case there is a great difference between intellectual success—success in work—and emotional success—success in human relationships. For many reasons we cannot predict genius; but we can predict mental backwardness.

Discussion. *A. Ylppö:* In some cases the prediction of development in children is relatively easy as in mongoloids, cretins and similar well defined abnormal conditions. But it is very difficult in children who have recovered from infections or traumatic lesions with some residual defects. Very often such defects are isolated residuals and do not prevent normal development in other respects. — *R. S. Illingworth:* I completely agree with professor Ylppö that very often in such cases the developmental possibilities are underestimated. We once treated a newborn child who was asphyctic and not breathing for 60 minutes. He nevertheless developed into a perfectly normal child.

Meeting April 23, 1960

M. Sulamaa: Tension Emphysema and Diaphragmatic Relaxation as Surgical Emergencies in Infants

It has not been easy to make a correct diagnosis and to decide the indications for operative treatment in cases of respiratory distress due to a tension cyst, lobar emphysema or a congenital diaphragmatic eventration. Since 1952 a gradually increasing number of cases have been brought to our surgical department at the same time as our attitude has become more active. The material comprises 10 cases of pulmonary tension emergencies and 12 cases of total diaphragmatic relaxation in infants under 9 months of age. The clinical picture was in most cases dominated by respiratory difficulty that had set in at a comparatively early age. Cyanosis was often present, particularly when the baby cried, and respiratory infections tended often to confuse the clinical picture. The symptoms were mostly progressive and they often occurred in bouts. The diagnosis of diaphragmatic relaxation is easy on X-ray pictures. The plication of the thin membranous diaphragm to the normal level through thoracotomy is a simple and mostly successful operation. Our material contains, however, also cases where respiratory and circulatory disturbances in newborn babies had been interpreted as asphyxia (without X-ray examinations) and a correct diagnosis and treatment had been missed until an autopsy was made. The X-ray diagnosis of lobar emphysema and tension cyst may be more difficult. Compensatory emphysema present together with atelectasis, pneumothorax and atelectasis caused by foreign bodies or staphylococcal pneumonias may produce differential-diagnostic difficulties. Typically the radiographs showed an emphysematous pulmonary lobe or tension cyst. The tension displaced the mediastinum to the opposite side and the other ipsilateral lobes were atelectatic. The interpretation of the clinical and the roentgenological signs was, however, often confusing. Resection of the affected lobe was followed by recovery in 4 cases. Some of the 6 fatal cases were referred too late or the symptoms were misinterpreted. Summarizing, a lobar emphysema or tension cyst and a total diaphragmatic relaxation in infants are surgical emergencies if there is respiratory distress and mediastinal displacement. The diagnosis of tension emergencies might be difficult, but an early resection gives excellent results. The diagnosis of relaxation is easy to make and the surgical correction with simple plication of the diaphragm through thoracotomy is mostly successful even in newborn babies.

K. V. Parkkulainen: **Present Treatment of Esophageal Atresia**

In the decade 1947–1957, 130 cases of esophageal atresia were treated at the Children's Hospital, Helsinki. The survival rate was appr. 25 per cent. In the years 1958–59, 51 additional cases were seen with a survival rate of 45 per cent. In consequence of the experiences gained, the main points of the present treatment are scheduled as follows: Preoperative diagnosis is made with esophageal catheterization and by plain thoraco-abdominal roentgen examination without contrast meal. No preoperative hydration. Emergency operation by extrapleural route without gastrostomy. A soft nasal feeding catheter through the esophagus into the stomach. Postoperatively no parenteral fluids, instead early feeding through the nasal catheter. Five per cent glucose dropwise by mouth. Oral feeding past the catheter begun on the tenth to twelfth postoperative day. Feeding catheter removed after three to four weeks. However careful the treatment of patients with esophageal atresia, there will always remain a certain mortality of ca. 30 per cent owing to unavoidable fatal complications (e.g. massive bilateral pneumonia) and to fatal associated anomalies. Using the schedule outlined above we think that our results will improve. This is shown by the last 25 cases in 1959–60, with 10 deaths, i.e. a 40 per cent mortality rate.

G. R. Wallgren and K. Salmenkivi: **On Acquired Gallbladder Diseases in Children**

Seven cases of acquired gallbladder disease in children treated at the Aurora Hospital surgical department between 1956–60 are presented. Of these 4 had gallstones and 3 had cholecystitis. The clinical course is described and a review is given of the literature where about 370 cases of this kind are described up to 1958. The incidence of gallbladder disease at autopsy of children has been reported as 0.28%. Special features of the disease in children are discussed and the conclusion made that it is by no means a rarity and should be kept in mind when making the differential diagnosis of abdominal pains in children.

L. Hjelt, M. Niemi and K. Kouvalainen: **On the Cholinesterase and Monoamino Oxidase of the Colon in Hirschsprung's Disease**

The cholinesterase and monoamino oxidase content of the colon were studied by histological methods in 12 operated cases of congenital megacolon. Acetyl cholinesterase was found in the ganglions of the myenteric plexus even when no ganglion cells were detectable. This suggests the presence of ganglion cells even in the narrow segment of the megacolon though they may be located more proximally than usually. Non-specific cholinesterase was found in diminished amounts in ganglions without ganglion cells. In the longitudinal muscle of the narrow segment a very pronounced non-specific cholinesterase activity was observed which might explain the dysfunction of this muscle. Monoamino oxidase was not found at all or in very small amounts in the ganglions of the narrow segment, whereas its activity was clearly increased in the dilated segment.

E. I. Wallgren: **Orthostatic Changes in Blood Pressure in Patent Ductus Arteriosus**

(To be published in Finska Läkaresällskapets Handlingar 1960.)

P. Louhivuori: **Recent Statistical Data on Infant Mortality in Finland**

The decrease in infant mortality in Finland from 1950 to 1959 has been remarkable. Having been 4.3% in 1950 it was only 2.4% in 1959. The decrease was most marked in infants over one week old. The decrease in perinatal mortality has been insignificant. The statistics clearly show the increasing tendency for the first week of life to contain the greatest risk of death for infants. Comparing rural and urban infant mortality the latter was shown to have been higher up to the late twenties, whereupon it has been

lower in urban areas, the difference being now 0.4%. Geographically there was a distinct difference between southern and northern parts of the country. Infant mortality varied from 2.7 to 3.1% in districts around the Polar Circle whereas it was from 1.7 to 1.9% in Southern Finland. Nativity has shown a steady decrease, having been 24.3‰ in the average between 1941 and 1950 it was 18.4‰ in 1959.

K. Lapinleimu: **Isolation of Enteroviruses in Finland in 1959–60**

Between September 1959 and March 1960 802 throat swabs and 440 faeces specimens were sent to the State Serum Institute for virus isolation. From the throat swabs an enterovirus (ECHO 9) could be isolated only once. The faeces specimens were from 361 patients. From these a virus could be isolated in 136 instances, the majority—106—being Polio I. ECHO 9 was isolated from 8 specimens, ECHO 11 from 2, ECHO 7 from 3 and in 17 cases the virus could not be identified. The geographical distribution was even for all viruses.

K. Penttinen, I. Rantasalo, L. Saxén and A. Ojala: **The Prevalence of Anti-ECHO 9 Virus Antibodies and its Correlation to the Occurrence of Congenital Malformations**

(To be published in Ann. Paediatr. Fenniae.)

I. Rantasalo: **On the Occurrence of Sonne Dysentery in Finland in 1959**

(To be published in Duodecim 1960.)

M. Kunnas: **A Statistical Study on Prematurity in Finland**

The material included 4692 prematurely born children divided into four groups according to birthweight: Group I (< 1250 g) 81 children; Group II (1251–1500 g) 264 children; Group III (1501–2000 g) 1080 children; Group IV (> 2000 g) 3267 children. Each group contained full-term children (2, 3, 30 and 190 children) as well as children with a relatively short gestational age, i.e. under 30 weeks (27, 48, 44 and 22 children). One thousand and fifty-five were children of mothers with toxaemia, they were evenly divided among the groups. Seven hundred and sixty-two mothers had other illnesses during pregnancy, of these 120 were infections, equally divided among the groups. There were 1160 twins of whom 31% (355) belonged to Group III. Thirty-six per cent (1762) of the children neither walked nor spoke at the age of one year. Such children made up half of Group I, in the other groups their percentage was 36, 41 and 34. The material included 23 children with cerebral palsy, 47 with malformations and 14 with severe mental retardation.

H. Tallqvist and H. R. Nevanlinna: **Iron Poisoning**

(To be published in Ann. Paediatr. Fenniae 1960.)

J. Visakorpi and S. C. Öhman: **Hartnup's Disease**

(To be published elsewhere.)

M. Paatela: **On the Morphology of the Nephron**

The morphology of nephrons in children of various age groups was studied with the microdissection method. Normal as well as diseased kidneys were included. The nephrons of prematures are short and rather undifferentiated in structure. Those of the full-term newborn and of infants resemble those of the adult in pattern but are still considerably shorter. An attempt to localize the lesions in the epithelial structure of the nephron in some renal disorders was shown. The material consisted of 15 cases of congenital nephrosis, a few cases of lipoid nephrosis and one case of each of renal acidosis, acute tubular necrosis, nephrocalcinosis, Lignac-Fanconi's disease and Hartnup's disease. The study is still in progress.

K. Kouvalainen: **Experimental Congenital Nephrosis**

An attempt to produce congenital nephrosis in rats by means of substances known to produce nephrosis is described. The administration of aminonucleoside of Stylomycin (Lederle) to rats during pregnancy caused death of the offspring 1–4 days after birth. In their kidneys slight nephrotic changes could be detected. I^{131}-labeled globulin of anti-ratkidney serum passed through the placenta as of the rat soon after intravenous injection it could be demonstrated in great amounts in the placenta and in the fetuses. Studies on the effect on the offspring of administering nephrotoxic serum to pregnant rats were outlined as well as studies on the electron-microscopic changes produced in fetal ratkidneys by aminonucleoside.

T. A. Miettinen and J. Perheentupa: **Serum Protein-bound Carbohydrates and Mucoproteins in Normal Newborns and in Pathological Neonatal Jaundice**

Total protein, mucoprotein, protein-bound hexose, hexosamine, sialic acid and fucose were studied in sera of normal and pathologically icteric newborns. Corresponding assays were made in mothers of normal infants at delivery. In this report sialic acid (SA) represents the protein-bound carbohydrates. The values for SA and mucoprotein (Mp) in maternal sera were about two times higher than those in umbilical cord blood. A significant increase was observed during the first week of life especially if the values are expressed per protein. In erythroblastosis fetalis due to Rh-immunization SA and Mp had a tendency to low values in infants with low serum bilirubin. The decrease of Mp here seemed to be greater than that of SA. Newborns with ABO-incompatibility and cases of pronounced jaundice with no detectable immunization had normal or slightly elevated values. A significant increase of SA and Mp was observed in those individuals of the above-mentioned groups, who had elevated directly reacting bilirubin suggesting intrahepatic biliary obstruction. The generally reported decrease of Mp in hepatitis was not confirmed in this study as all cases of hepatitis diagnosed clinically and with current laboratory methods had normal or elevated concentrations of SA and Mp. Further studies on this question are in progress. Markedly high values were detected in one case of biliary atresia, the diagnosis was verified at operation.

T. Kantero, Helsingfors

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BOOK REVIEWS

Grundler-Seige: **Kinderheilkunde–Diagnostik–Therapie–Prophylaxe.**

Hippokrates-Verlag G.m.b.H. Stuttgart 1959. Price DM 29:—.

Prof. Grundler and Dr. Seige have published a manual of pediatrics, about 800 pages, in pocket-size. For medical students and general practitioners this volume is a valuable consultant source in checking the knowledge they have from textbooks. Each chapter also has a short bibliography.

Ole Wasz-Höckert, Helsingfors

Child Development. An International Method of Study. Edited by *Frank Falkner*.

S. Karger. Basel/New York. 1960. Price sfr. 50:—.

This is the first comprehensive report of the Growth Study performed in London, Zürich, Paris, Bruxelles, Stockholm, Dakar, Kampale and Louisville sponsored by the International Children's Centre in Paris under the direction of Professor Robert Debré. Professor Debré has written forword and Doctor Falkner, who is the co-ordinator of the study, an introduction and the history of the development of this international study. Hiddley (London team) discusses, in General Considerations, the longitudinal study contrasted to the cross-sectional study of growth and problems related to the techniques. Falkner (Louisville team) presents some of the methods and Graffar (Bruxelles team) gives an interesting report of the social study and Hindley of the psychological investigations carried out. Falkner describes the somatic investigations. The special problems and the adaptation of the methods used with regard to growth study in underdeveloped countries are stressed by Sénécal (Dakar team). Dean (Kampala team) devotes a chapter to the effects of malnutrition on the growth of young children. Ferrel (Louisville team) and Karlberg (Stockholm team) have contributed a discussion on practical problems regarding recording and analysing of the data of research. The last 100 pages of the book contain appendices related to the methodology and the questionnaires used in this important international study.

The thyroid gland

British Medical Bulletin. Vol. 16 No. 2. May 1960. Price £ 1.

In the series of review articles published by the Medical Department of the British Council as the British Medical Bulletin, this year's May number is devoted to problems concerning the thyroid gland. Sir Charles Harington says in his introduction that during the past ten years research on the thyroid gland has developed in many directions and to a much greater extent than might have been foreseen. The papers contained in this number indeed reflect these facts.

The contributors are leading British specialists in the field of thyroid research and most of the more important and recent work is covered. Several articles have a special interest from the paediatric point of view. To these belongs the paper on the passage of thyroid hormone from mother to foetus by Osorio & Myant and "Sporadic goitrous cretinism" by McGirr. Trotter is dealing with the problem of deafness associated with thyroid dysfunction. Of great general interest are the papers on thyroid auto-immunity, biosynthesis of the thyroid hormones and genetic factors in thyroid diseases. It is not possible to mention all the contributions in a short review but it may be

said in summary that the high standard of the content of this number makes it easy to recommend.

C. G. Bergstrand, Stockholm

Daniels Stowens: Pediatric Pathology

The Williams and Wilkins Company, Baltimore 1959, 676 pages. Price Sw. crowns 120:90.

As stated in the preface "this is a work devoted to anatomic appearances with respect to diseases of childhood". These diseases are discussed in 35 chapters.

It is not easy to write a book about pediatric pathology to include everything that is important without making the book too extensive and too expensive. On the other hand, a book only listing the diseases and not describing and discussing them would be of little value. It is impossible to write a book about pathology and stating opinions every pathologist would agree with. In this book the author has not only described the pathology but also has given his personal opinion in several instances. This makes it probable that opinions of the readers will differ in several areas from the author's opinions but as is generally known pathologists seldom agree on every question. There are some statements without proof, and the reader would be interested to find out more about the basis of the opinion of the author, but this is not always possible since references do not contain the name of the original paper. However, it is good for a reader interested in pediatric pathology to see opinions differing from those of his own. Further this makes the book fresh and interesting and shows that the author has done some thinking and not only collected facts.

The book is warmly recommended to pathologists who deal with its pediatric subspecialty and for pediatricians interested in knowing more about the anatomy of the diseases of children.

E. K. Ahvenainen, Helsingfors

ANNOUNCEMENT

Teratology Society

For several years scientists interested in basic problems of congenital malformations have held informal conferences in which questions of common interest were discussed. Anatomists, biochemists, embryologists, geneticists, obstetricians, pathologists, pediatricians, plastic surgeons and others attended these conferences which were in part supported by the Association for the Aid of Crippled Children, New York, N.Y., and the Human Embryology and Development Study Section of the National Institutes of Health. With the increased interest in recent years in this area, it was felt that there was a need for a Society to hold regular meetings in which investigations concerned with etiology and morphogenesis of congenital malformations could be presented and discussed. Following the fourth teratology conference, which was held at the Memorial Sloan-Kettering Cancer Center in New York City and attended by 76 scientists from Canada, England, France, Germany and the U.S.A., "The Teratology Society" was formed for the purposes outlined above. Josef Warkanay, M. D., Cincinnati, was elected President. Inquiries about The Teratology Society should be directed to Dr. Marjorie M. Nelson, Department of Anatomy, School of Medicine, University of California, San Francisco 22, Calif.

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Hashimoto's Disease in Childhood

A Clinical and Immunological Study of Five Cases

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In 1912 Hashimoto (10) described a goitre characterized by the formation of lymph follicles in the thyroid, an increase in lymphocytes, and subsequent marked fibrosis and destruction of the thyroid follicles. Almost 20 years elapsed before any new cases were reported, since then, however, a great number have been communicated. At present the disease is not considered to be exceptionally uncommon; in the material collected by Statland, Wasserman & Vichery (19), Hashimoto's disease was found to be the cause of 1.4 % of all thyroidectomies. The etiology, which earlier was a matter of controversery, is still obscure, although the demonstration of cytotoxic antibodies against thyroid tissue in serum from patients with Hashimoto's disease gives strong evidence for the participation of an auto-immunisation mechanism in the pathogenesis of the disease (5, 13, 15, 16, 17, 18, 22).

The course of the disease is a slow one. Usually an enlargement of the thyroid will appear, in some cases it may be rather insignificant, in others so pronounced that it will cause compression. Simultaneously the gland becomes firmer. There is no pain. As shown by various tests, thyroid function is already impaired in the early stages of the disease, but hypothyroid symptoms appear only gradually.

Hashimoto's disease affects mainly elderly women, but it may also appear in younger age groups and in men. It is said to be rare in childhood (23). In a study of 605 cases 3 were below the age of 10 years (23). All were girls, 8 or 9 years old. In other studies which included children only girls have been reported as being affected (9, 19).

In this communication the clinical and other findings in Hashimoto's disease as it affects children will be reported. Five patients have been studied, two of them are boys.

Methods

BMB was estimated according to the method of Karlberg (11).

PBI was estimated using the method of Baker, Humphrey & Soley (1).

Serum cholesterol was estimated according to the method of Pearson, Stern & McGavack (12).

Thyroid uptake of I^{131} *after 24 hours*

was measured at Radiumhemmet, Karolinska Sjukhuset, Stockholm by means of a sodium iodide scintillation counter, provided with a wide-angle lead collimator. The crystal-to-skin distance was 30 cm and the influence of secondary radiation was reduced by a 2 mm lead filter. The standard solution of I^{131} was measured in a fluid volume of 20 ml contained in a cylindrical vessel of 3.7 cm diameter (6).

Purified thyroglobulin was prepared by precipitation with $(NH_4)_2\ SO_4$ from saline extracts of fresh thyroids removed at operation and of post-mortem material according to the descriptions given by Derrien, Michel & Roche (4).

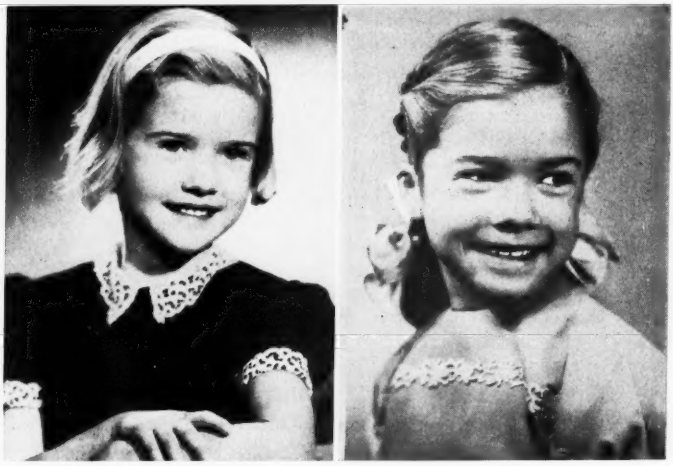
Precipitin reactions. The techniques applied were those of gel-diffusion according to Ouchterlony using 1.5 % agar in physiological saline as a medium and small plastic containers for the reagents. The containers were refilled several times (14).

Immunophoresis. Immunelectrophoretic studies on supporting glass plates, 5×8 cm large, were carried out according to the method of Williams & Grabar (8). The electrophoretic separations were performed in a barbiturate buffer of pH 8.3 and with an ionic strength of 0.05.

Haemagglutination tests. The indirect haemagglutination technique of Boyden (3) was used in all experiments and carried out as follows: In order to inactivate complement all sera were incubated at 56°C for 30 minutes. In addition, heterophile antibodies were removed by absorption with washed sheep erythrocytes. Sheep erythrocytes were washed once in physiological saline and three times in physiological saline buffered with 0.15 M phosphate buffer and finally diluted in this buffer to a concentration of 50 %. An equal volume of tannic acid in a dilution of 1:40,000 was then added dropwise and the suspension was incubated at 37°C for 20 minutes. After centrifugation for 5 minutes at 1500 rpm and subsequent washing in buffered saline, 1 volume of the "tanned" cells was incubated at 37°C for 30 minutes with 3 volumes of the thyroid extract. The cells were then centrifuged off (5 minutes at 1500 rpm) washed once in saline and were finally suspended in buffered saline to give a final concentration of 0.25 %. Serial dilutions of the sera to be tested were made in buffered saline. One half ml of each dilution was mixed in a test tube with an equal volume of sensitized cells, or only of "tanned" cells or only of washed cells (controls). For stabilisation 0.1 ml of rabbit serum was added to each tube. The tubes were thoroughly shaken and incubated at 37°C for two hours, and shaken occasionally. The degree of agglutination was recorded macroscopically against an illuminated background. *Complement fixation test:* The micromethod of Belawyian was used in all experiments (2).

Case Reports

Case 1. Girl. This patient was sent to the Hospital because of nanosomia at an age of 12 years. When she was 7 years old her height was 118 cm but since then she had only increased 5 cm. Except for some asthmatic troubles she had, according to the parents, been in good health. However, by careful questioning it was discovered that she had changed in appearance during the last 5 years, developing coarser features with dry hair and skin and also becoming more indolent. On admission her height was 123 cm. She had a hypothyroid appearance, a square build with coarse features, cold dry skin, and straight hair. The gait was rocking. No secondary sex characteristics had developed. The thyroid gland was not palpably enlarged. She had normal blood values but the sedimentation rate was moderately elevated, 30 mm/h. The laboratory investigations revealed hypothyrotic values, the PBI was 1.4 γ %, serum cholesterol 872 mg % and an iodine uptake by the thyroid gland of only 1.4 % after 24 hours, which increased only to 4 % after 24 hours following TSH-stimulation. The BMB was depressed to −42 %. The bone centres corresponded to an age of 9 years and there were slipping epiphyses on both hips. The amount of growth ho-



a b

Fig. 1. Photographs of Case 1 at different ages illustrating the change in appearance during the course of the disease. a/5 years (2 years before the onset of the disease). b/10 years.

mone in the blood was normal. On intelligence testing (Grace Arthur Perf. II), she achieved an IQ of 67. A diagnosis of acquired hypothyrosis was made, Hashimoto's disease was discussed, but as she did not have any palpable enlargement of the thyroid, and permission to perform a biopsy could not be obtained, the cause remained unknown. She was treated with desiccated thyroid and the signs of hypothyrosis disappeared quickly. At a check up a year later she had grown 15 cm and she did not show any symptoms of hypothyrosis. The PBI was 7.1 γ %, serum cholesterol 242 mg % and BMB +5 %. At this time at a chronologic age of 13 years her bone centres corresponded to an age of 12 years. However, the IQ had not improved. She has continued to take desiccated thyroid and her condition has remained good, though at the age of 14 years a moderate enlargement of the thyroid gland was palpable. At the age of 15 years the immunological investigations which gave support to the diagnosis of Hashimoto's disease were carried out. The precipitin reaction was positive and the haemagglutination test was positive to a dilution of 1:100,000. However, the complement fixation test was negative in this case.

Case 2. Girl. This girl came to the Hospital at the age of 13 years because she had become more lethargic during the last year. In spite of this her results in school were as good as before. Her general appearance had become coarser during this time. During the two preceding years she had grown only 3 cm. On admission her height was 141 cm. She had a moderate hypothyroid appearance with dry skin and hair, and cold extremities. She had not yet reached puberty. The right thyroid lobe was moderately enlarged, somewhat firmer than ordinary thyroid tissue. The laboratory investigations showed values as in hypothyroidism. Sedimentation rate was 35 mm/h. PBI 2.0 γ %, serum cholesterol 442 mg %. The iodine uptake of the thyroid was decreased; 15 % after 24 hours. Her number of bone centres corresponded well to her age. She was treated with desiccated thyroid and made a prompt recovery. One year later she had increased 12 cm in height. The enlargement of the right

Fig. 1. c/ 12.5 years (immediately before starting treatment).

Fig. 1. d/ 14.5 years (after 2 years of treatment).

lobe remained unchanged. Her laboratory values were now normal, PBI 6.2 γ % and serum cholesterol 142 mg %. The immunological tests performed at this time showed a haemagglutination titre of 1:100,000 and a positive precipitin reaction. In this case as well the complement fixation test was negative.

Case 3. Boy. In this boy an alopecia areata began at the age of 8 years. Previously he had enjoyed good health and had developed normally. The alopecia was treated with different hair tinctures, and cortisone ointment without result. Because of the increasing loss of hair he was admitted to the Hospital at the age of 13 years. During the previous 4 years he had been cold and his body structure had become coarser. However, his school results hade been very good, in fact he had no other interests.

On physical examination he appeared to be a fairly short 13 year old boy (the height was only 139 cm). He had a somewhat dry skin with a touch of yellow and a slight hirsutism. The eruption of the permanent teeth was delayed. He did not show any secondary sex characteristics. The thyroid gland was moderately enlarged without any roentgenological signs of compression or dislocation of the trachea or oesophagus. Laboratory investigations: Sedimentation rate 35 mm/h; PBI 2.9 γ %; serum cholesterol 387 mg %; BMB −30 %. The iodine uptake of the thyroid was depressed; 13 % after 24 hours. The same value was obtained after stimulation with TSH. The 24 hour urinary excretion of 17-keto steroids was 5.6 mg. The value for corticosteroids was 5.2 mg. The number of bone centres corresponded

well with the age. An intelligence test, (Terman-Merill), showed an IQ of 127. A biopsy was taken from the thyroid gland. The examination revealed tissue with atrophic changes, showing an increase in connective tissue with a diffuse infiltration by lymphocytes and plasma cells. The findings supported the diagnosis of Hashimoto's disease. Treatment with desiccated thyroid was started at this time and during the following 1½ years his height increased 20 cm, so that he is now well within the normal range for his age. The yellow colouring of the skin disappeared after some months of medication. At that time the laboratory values became normal with a PBI of 5.4 γ %, a serum cholesterol level of 161 mg % and a BMB of +25 %. He has remained on desiccated thyroid and is doing well so, though his alopecia does not seem to have changed. When he had been treated for almost 2 years immunological studies were performed. These gave further support to the diagnosis of Hashimoto's disease. The precipitin reaction was positive. Haemagglutination occurred up to a dilution of 1:200,000. The complement fixation test was positive in a titre of 1:4096.

Case 4. Boy. In an Infant Welfare Centre this 5-year-old boy was found to have a goitre, because of which he was admitted to the Hospital for investigation. He had always been in good health and his development had been normal both physically and mentally. On admission he did not show any signs of hypothyreosis, he was alert, his height (105 cm) was within normal limits for his age, his skin and hair were normal. His thyroid gland was moderately enlarged and somewhat firm. The trachea was dislocated about 1 cm to the right on X-ray, but there was no compression. The number of bone centres were within normal limits for the age. He did not show any epiphyseolysis but the centres of the epiphyses in the hips were very irregular. An intelligence test, (Terman-Merill), showed an IQ of 101. Laboratory investigations: sedimentation rate 18 mm/h; PBI 2.3 γ %; serum cholesterol 311 mg %; BMB −7 %. The iodine uptake in the thyroid gland was increased; 66 % after 24 hours. With these results a congenital enzymatic defect of the thyroid could not be excluded. A biopsy was denied by the parents. The immunological tests, however, supported a diagnosis of Hashimoto's disease. The precipitin reaction was positive. The haemagglutination titre was 1:200,000 and the complement fixation test was positive in a titre of 1:1024. The patient has since then been treated with desiccated thyroid and has not developed any hypothyreotic symptoms. His thyroid gland has decreased somewhat in size but has become still firmer. The laboratory values became normal after a few months and have remained so during the 2 years of observation.

Case 5. Girl. Because of a congenital deafness of unknown origin this girl was sent to a boarding school for deaf children. At the age of 12 years she was observed to have a goitre. At that time an X-ray was taken. This showed a slight compression of the trachea. The PBI was normal; 5.3 γ %. The BMB was +2 % and the serum cholesterol level was 227 mg %. During the following year the goitre increased in size. She was therefore admitted to the Hospital for investigation at the age of 13 years. At that time she complained of difficulties in swallowing. The physical examination revealed a normally developed 13-year-old girl, who had not yet reached puberty. Her length was within normal range for her age. She did not show any signs of hypothyreosis, her skin was moist and warm. She had a goitre about the size of an orange. The gland was diffusely enlarged with some predominance of the right lobe which was rather firm. An X-ray performed at this time showed an increasing compression of the trachea as compared to that shown the year before. The bone centres were normal in number for her age. Sedimentation rate was 19 mm/h. At this time the PBI had decreased to 1.7 γ %, and the serum cholesterol level was elevated at 456 mg %. She had an increased iodine uptake in the thyroid, 63 % after 24 hours. The immunological tests demonstra-

ted the presence of circulating antibodies aginst thyroglobulin and thyroid extracts, the precipitin test was positive, the haemagglutination test was positive in a titre of 1:100,000 as was the complement fixation test in a titre of 1: 512. Because of the suspected autoimmune pathogenesis of Hashimoto's disease treatment with cortisone was tried. She was given a dose of 75 mg prednisolone daily for three weeks and was then kept on a dose of 50 mg prednisolone daily for 2 months. Following this the goitre diminished to about ¼ of its original size, the PBI had risen to 4.4 γ %, the serum cholesterol level was normal at 178 mg % as was the thyroid uptake of I^{131}. The dose of prednisolone was later gradually decreased but now when the dose is only 5 mg daily the PBI has sunk to subnormal levels.

Discussion

In this series 2 of the 5 patients are boys, a finding which does not agree with the common sex distribution, as Hashimoto's disease is very seldom seen in males. However, it is difficult to compare a group of children with the findings in adults. From our small number of cases it is, of course, not possible to determine the sex distribution in children.

The age at which the patients were first seen by us varied from 5 to 13 years. None of them had at that time reached puberty. The age of onset is uncertain. It has been calculated by studying old photographs of the patients and above all by studying the growth and development charts from the infant welfare centres and schools. The long interval ensuing from the calculated age of onset and the age when the patients were first seen, indicates that the hypothyreotic symptoms were not noticed by the families. This is also demonstrated by the fact that in many cases nonhypothyreotic symptoms brought the patients to hospital. Three of the patients showed clear evidence of hypothyreosis with coarse features, hyperkeratosis, cold extremities, coarse voice, and lethargy. These three were all of short stature. Their growth and development had practically ceased from the moment of onset of the disease. In those two patients where the growth did not seem to be affected, the disease had obviously been of shorter duration, judging from the history and the laboratory investigations. None of the patients suffered from constipation, a frequent symptom in hypothyreosis. Of the 3 children with a long history and marked hypothyreotic symptoms only one was mentally retarded with an IQ of 67. The other children, despite their lethargy, were not retarded. On the contrary they belonged in the better third in their school classes. Nor did the hypothyreotic state, developing late in childhood, seem to affect the number of bone centres to any great degree. The slipped epiphyses which occurred in one patient and seemed to be developing in another patient may be of greater diagnostic value (U. Rudhe, personal communication).

The goitre was of variable size, in one case insignificant, while in another case it gave rise to symptoms of compression. None of the cases underwent thyroidectomy. Permission for biopsy could be obtained only in one case and in this case the diagnosis of Hashimoto's disease was supported by the histological picture.

The laboratory findings were the same as those in adults; with a moderately increased sedimentation rate, a decreased PBI, a depressed or normal BMB, an elevated serum cholesterol level and

TABLE 1. *Summary of the Clinical Data in the Patients with Hashimoto's Disease.*

Case	Sex	Probable age at onset	First seen in hosp. Age	Cause	Sed.rate mm	PBI γ%	BMB %	Cholesterol mg %	Iodine uptake by the thyreoid
1	♀	8 years	12 y	Nanosomia	30	1.4	−42	872	decreased
2	♀	12 „	13 y	Lethargy	35	2.0	−32	442	decreased
3	♂	9 „	13 y	Alopecia	35	2.9	−30	387	decreased
4	♂	5 „	5 y	Routine check up	18	2.3	−7	311	increased
5	♀	12 „	13 y	Goitre	19	2.6	+2	456	increased

TABLE 2. *Summary of the Immunological Findings in the Patients with Hashimoto's Disease.*

Case	Sex	Presence of precipitating antibody	Haemagglutination titre	Complement fixation
1	♀	+	1/100,000	Negative
2	♀	+	1/100,000	Negative
3	♂	+	1/200,000	Positive
4	♂	+	1/200,000	Positive
5	♀	+	1/200,000	Positive

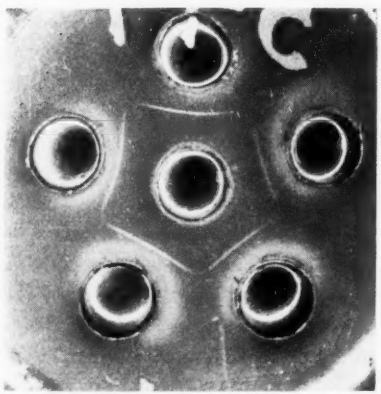


Fig. 2. Dark field photograph of gel diffusion plate on agar medium (Ouchterlony technique). Thyroglobulin in the central basin is allowed to react with five different sera from Cases 1, 2, 3, 4 and 5 in the peripheral basins. From the continuity of the precipitate it can be concluded the components of serum precipitated by thyroglobulin are identical in the five cases.

varying uptake of I^{131} in the thyroid. The variation of the iodine uptake in the gland depends on the ability of the gland to react to thyrotropic stimulation; i.e. early in the disease the thyroid tissue is not yet so badly damaged that it cannot respond to stimulation, whereas later in the disease the parenchyma is too destroyed to respond to any stimulation.

The immunolgical findings (cf. Table 2) give strong support to the diagnoses. With a gel diffusion technique it was possible to demonstrate precipitating antibodies against purified thyroglobulin in all cases (Fig. 2). The same results were obtained when a crude saline extract of normal thyroid tissue was used as an antigen. It is seen from Fig. 3. that the precipitating antigen of the thyroid extract moves with the same electrophoretic mobility as thyroglobulin.

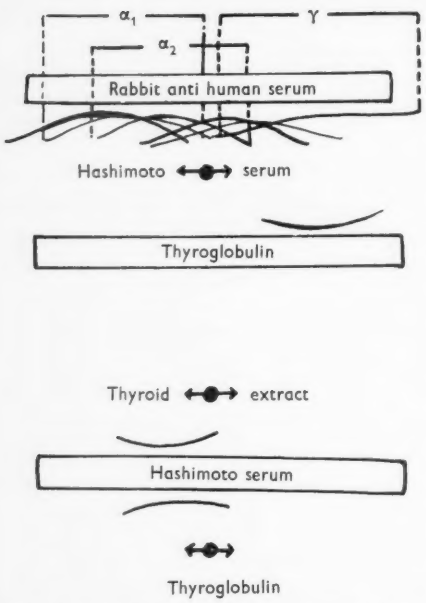


Fig. 3. Drawing of immunelectrophoretic plate. In the upper central basin serum (from Case 2) is electrophoretically separated and allowed to react with an anti-human serum and purified thyroglobulin. It is seen that thyroglobulin precipitates a component of the γ-globulin fraction of the serum of the patient. In the two central basins below, a saline extract of normal human thyroid gland and purified thyroglobulin are separated and allowed to react with serum from Case 2. It can be seen that thyroglobulin moves with the same electrophoretic mobility as that fraction of thyroid extract which is precipitated by the serum. The electrophoretic mobility is the same as that of the α_1 and α_2 globulins of serum.

It is also shown that thyroglobulin and the precipitating antigen of normal thyroid tissue move with the same electrophoretic mobility as the α_1- and α_2-globulins of normal human serum (as has been earlier demonstrated by Roitt, Campbell & Doniach (16)). Using Boyden's haemagglutination method it was possible to demonstrate the presence of haemagglutinating antibodies in serum dilutions up to 1:100,000 and 1:200,000 in all cases. In this investigation thyroid extract was used as antigen. Complement fixing antibodies could, however, not be demonstrated in more than 3 of the cases. This is somewhat confusing but may be due to the fact that the patients, investigated, had been treated for a long time or that the antigen used was very weak. The antigen responsible for the complement fixing antibodies is only found in considerable amounts in thyrotoxic thyroid tissue. The thyrotoxic thyroid tissue used in our preparation was taken from patients who had undergone operation after previous iodine treatment. This treatment might have altered the antigen.

Precipitating and haemagglutinating antibodies have also been demonstrated in patients with thyroid disorders other than Hashimoto's disease. As shown by Roitt & Doniach (17) antibodies against thyroid extract could be demonstrated in 98 % of patients with Hashimoto's disease, 83 % of patients with spontaneous adult myxoedema, 64 % of thyrotoxics, 33 % of patients with non toxic collagen goitre, 68 % of patients with subacute thyroiditis and 29 % of thyroid malignancies. The highest antibody titers are nevertheless found in Hashimoto's disease. The fairly high haemagglutination titres in the cases presented and the ability to demonstrate antibodies by the gel diffusion technique support the diagnosis in spite of the negative complement fixation test.

The demonstration of circulating antibodies to thyroid tissue antigens in patients with Hashimoto's disease will not in itself prove that these antibodies are ultimately responsible for the pathogenesis of the disease. The cytotoxic effect of the ci-

culating antibodies on human thyroid tissue in tissue-cultures has recently been demonstrated by Pulvertaft, Doniach, Roitt & Hudson (13). Rose & Vitebsky (18) have also been able to produce a disease with the histopathological characteristics of Hashimoto's disease by injecting rabbits with their own thyroid tissue. The cytopathogenic effect of the circulating auto-antibodies seems therefore to be ascertained. However, the question still remains: what mechanisms are responsible for the induction of the auto-immune processes? The thyroid antigens belong to the same group of native auto-antigens as certain proteins of the lens, brain tissue and spermatozoa, antigens to which a tolerant state has not been achieved during embryonic life due to the fact that they have never been in contact with the anti-body forming system. Experimental studies by White (22) using a fluorescein-labeled antibody technique have given definite indications that there occurs, in Hashimoto's disease, a leakage of thyroid antigens into the circulation thus affording the conditions needed for a production of specific antibodies. Another question then arises: what is the cause of this leakage of thyroid antigens? Many factors have to be considered in this context. A subclinical thyrotoxicosis has been proposed to be the inducer. Though the age and sex distribution would agree with this assumption, clinical evidence is so far lacking. The histological picture of the goitre is not inconsistent with a chronic infection, possibly of viral origin. It is therefore possible that the initiating mechanism is after all an infection, and that infection ultimately is responsible for the pathogenesis.

The treatment of Hashimoto's disease has always been substitution therapy. Four of our patients have been continuously treated with desiccated thyroid in individually balanced doses. During this treatment their hypothyreotic features have quickly disappeared and their growth increased rapidly, so that at present all are within the normal range for their ages. Euthyroidism has been restored in all cases as revealed from the laboratory investigations. As we are dealing with an auto-immune disorder where the circulating auto-antibodies obviously are responsible for the destructive processes in the thyroid parenchyma cortisone treatment might be expected to be a valuable form of therapy. As can be seen from the case reports only one patient has been given steroid treatment (Case 5). This patient was obviously caught very early in the disease as indicated by the increased iodine uptake and the absence of more advanced clinical signs of hypothyreosis. However, her antibody titers were fairly high with a haemagglutination titer of 1:200,000. As there was apparently no definite destruction of the thyroid tissue a restoration of the thyroid function could perhaps be expected if the production of thyroid antibodies could be supressed at an early stage of the disease. During steroid treatment there was a marked fall in the antibody titres, from 1:200,000 to 1:100, simultaneous with a complete restoration of the thyroid function and an almost complete disappearance of the goitre. It is tempting to ascribe the restoration of the thyroid function to the depressed production of specific antibodies. One must bear in mind, however, that it could be a mere

coincidence due to two different actions of steroids: the well-known inhibitory effect on the production of antibodies and a more nonspecific anti-inflammatory effect on the goitre. A third possibility should also be taken into consideration, namely, that a spontaneous remission could have occurred during the steroid treatment. The latter alternative seems, however, most unlikely as the thyroid function showed signs of impairment when the steroid dose was lowered below 10 mg daily.

However, wider experience with steroid treatment has to be obtained before it can be recommended for more extensive use in early cases of Hashimoto's disease. High doses over a long time seem to be necessary and during this time the patients are exposed to all the deleterious side-effects of cortisone. The conventional substitution therapy is the only available therapy in more advanced cases and must be regarded as a safer method of treatment even in early cases.

Summary

Hashimoto's disease has long been considered a rare disease in childhood. A series of 5 children (3 girls and 2 boys) with well established diagnoses is presented. The age of onset varied from 5 to 12 years. The children have, in addition to the investigation of the thyroid function with conventional methods, also been investigated with immunological methods, with regard to circulating antibodies against components of thyroid tissue. Circulating antibodies in high titres have been found in all cases. The pathogenetic role of the autoimmunisation, which evidently occurs in this disease, is briefly discussed. Assuming that the autoimmunisation is of fundamental pathogenetic significance one of the patients who obviously was caught very early in the disease was treated with steroids. During this treatment there was a marked fall in the antibody titres simultaneously with a complete restoration of the thyroid function, indicating that there is a causal relationship between the suppression of the production of specific antibodies and the restoration of thyroid function. The other four patients have been successfully treated with desiccated thyroid in individually balanced doses. At the present stage of knowledge this therapy is the only available one in more advanced cases and must also be regarded as safer, even in early cases.

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Gamma Globulin in the Prevention of Cross Infections in Hospitalized Infants

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Infections contracted in hospitals are common, especially in childrens' hospitals and nurseries. There have been reports of sulfa or antibiotics as more or less valuable prophylactics, which from other points of view are not desirable (2, 4). As gamma globulin contains antibodies against infective agents of both viral and bacterial origin, and as one of us (Lundström, 5), had noted a probable protective effect of gamma globulin against upper respiratory infections in a childrens' home, it seemed desirable to undertake a controlled study to investigate the possibility of increasing the resistance of infants against hospital infections with gamma globulin.

Material and Methods

The investigation was planned so that every second infant received an intramuscular injection of gamma globulin on admission to the nursery, every other infant was given a placebo preparation in a dose of 1 ml per kg bodyweight.[1] The type of preparation administered was unknown to the investigators as well as to the nursery staff until the study was completed. Infants remaining more than three weeks in the ward, received a new injection at the beginning of every three week period, the preparation and dosage being the same as on admission. On admission, and every three weeks, nose and throat swabs were cultured for bacterial pathogens. When infections occurred isolation of such pathogens was attempted as well. Routine examination for viral pathogens was not performed. Cross infection is defined in this study as infection occurring after the third day of hospitalization. It includes upper and lower respiratory infections, infectious dyspepsias, pyodermic conditions, otitis, and urinary infections.

The prematures were nursed in two separate rooms, usually 2–4 patients at the same time in each room. Other infants were nursed in a common large room where airborne infections might spread easily. In this room usually about 15 infants were treated simultaneously.

[1] AB KABI, Stockholm, has supported this study and supplied the preparations used, namely a 12 per cent solution of gamma globulin, derived from retroplacental blood, and the placebo, consisting of a water solution containing sodium carboxymethylcellulose 1.3 milligram, polysorbate 80 0.27 milligram, sodium chloride 9 milligram, and Edicol supra yellow 2 gamma per milliliter. Thus the preparations had the same slightly opalescent appearance.

Prior to computing the results, the following patients were excluded from the study: infants hospitalized less than four days, severely malformed infants, patients receiving blood transfusions, and patients given antibiotics or sulfa when receiving gamma globulin or placebo.

The investigation was performed from July 1st, 1955, through June 30th, 1956. As the number of prematures treated until then was very small and did not allow any conclusions the investigation concerning these was prolonged until June 30th, 1957.

A preliminary report of the findings has already been presented 1956 (1).

Results and Discussion

No local or general reactions as a result of the injections of the gamma globulin and the placebo were observed. The average time of hospitalization showed no significant difference in the two groups. The distribution of pathogenic bacteria was the same in both groups, pyogenic staphylococci and pneumococci being most commonly isolated.

As a rule, the level of gamma globulin is relatively high in the foetus from the seventh month of pregnancy on, as well as in the newborn (7, 8). After birth it drops to a minimum at about one month of age. After the third month the level slowly increases but remains relatively low throughout the first year of life. With these variations in mind the results are reported separately for infants of different ages.

Table 1 shows the incidence of infections in fullterm and premature infants up to four weeks of age. Ten out of 24 (42 per cent) full term infants developed cross infections in the gamma globulin as well as in the placebo group. Fourteen out of 33 (42 per cent) gamma globulin-treated and 12 out of 33 (36 per cent) placebo-treated prematures developed cross infections. No significant difference was observed between these two groups nor between full term and premature infants. During the first week of life 3 out of 17 full term infants in the gamma globulin group and 3 out of 15 in the placebo group had infections, whereas 2 out of 33 prematures treated with gamma globulin and 1 out of 33 treated with placebo developed an infection. Thus no significant difference between the gamma globulin and the placebo group was found as regards infections in the first week of life.

Table 2 presents the incidence of cross infections in infants more than 4 weeks of age on admission. Six out of 22 (27 per cent) infants treated with gamma globulin and 15 out of 28 (54 per cent) infants treated with placebo showed infections.

TABLE 1. *Cross infections among infants 0–4 weeks of age.*

	Gammaglobulin			Placebo		
	Number of infants	Number of infants with cross infections	Per cent	Number of infants	Number of infants with cross infections	Per cent
Prematures	33	14	42	33	12	36
Fullterms	24	10	42	24	10	42

TABLE 2. *Cross infections among infants more than 4 weeks of age.*

Gammaglobulin			Placebo		
Number of infants	Number of infants with cross infections	Per cent	Number of infants	Number of infants with cross infections	Per cent
22	6	27	28	15	54

TABLE 3. *Duration of cross infections among infants more than 4 weeks of age compared with total time of hospitalization.*

	Number of infants	Total number of weeks in nursery	Weeks with infections	Mean value of the individual ratios: *weeks with infections* total number of weeks	Dispersion of these ratios
Gammaglobulin	22	65	7	0.10	0.22
Placebo	28	73	30	0.35	0.36

The difference between the incidence in the two groups is almost significant ($p = 0.05$).

Table 3 shows the means and the standard deviation of the individual ratios between number of weeks with cross infection and total number of weeks in the nursery, for infants more than 4 weeks of age on admission. In the series of infants receiving gamma globulin 22 infants were hospitalized for a total of 65 weeks, showing infections in 7 weeks, the mean value of the individual ratios being 0.10. Corresponding figures in the placebo group are 28 infants hospitalized 73 weeks, showing infections in 30 weeks, the mean value of the individual ratios in this group being 0.35. The difference is significant ($p < 0.01$).

In infancy, susceptibility to infections may be influenced by the kind of feeding (6). Therefore, the following data are given. The infants up to four weeks of age were routinely fed with human milk, mainly pasteurized. Fourty-four per cent of the older infants were fed similarly, whereas the others received simple cow's milk formulas, mashed vegetables and juices added according to age. Vitamins A and D were given routinely. In the placebo group of infants more than 4 weeks old, the incidence of infections did not differ between the infants fed with human milk and those fed with cow's milk. In the gamma globulin group, however, only one out of six infants with infection was fed with human milk, whereas five were artificially fed, the number of not infected comprising ten breast-fed and six bottle-fed infants. This shows a difference which, without being significant, gives an impression of protection by gamma globulin in the breast-fed but not in the bottle-fed infants.

The conclusions to be drawn from the aforementioned data are, that in this study

gamma globulin does not seem to increase the resistance against infections in the first weeks of infancy, this being applicable to full term infants as well as to the prematurely born. From the data given in Tables 2 and 3 it might be concluded that gamma globulin to some degree has had a protective effect against cross infections occurring in the nursery, for the period of investigation, in infants more than four weeks old, e.g. a period when, as a rule, a physiological hypogammaglobulinaemia has developed.

Summary

(1) Human gamma globulin, 12 per cent, 1 ml per kg bodyweight, was administered to every other full term or premature infant admitted to the Infants' Nursery of the Hospital, alternate patients receiving a placebo preparation, for one year; the period of investigation being prolonged in the case of the prematures for another year. The incidence of cross infections in the infants was studied, the authors being unaware of the identity of the preparations used until the study was completed. The investigation comprises 98 full term patients and 66 prematures. (2) During the first week and even the first four weeks of life gamma globulin apparently did not effect any added protection against cross infection in either full term or premature infants. (3) In infants more than 4 weeks old, gamma globulin seemed to prevent cross infections to a certain extent, the incidence of infections being significantly lower in the group of infants treated with gamma globulin. This might be explained by the occurrence of a physiological hypogammaglobulinaemia in this age group, possibly corrected by the treatment. (4) Apparently, feeding with pasteurized human milk as opposed to cow's milk did not influence the occurrence of cross infections in the control group, whereas the possible protective benefit of gamma globulin was more prevalent among the infants fed human milk.

Acknowledgement

The authors are indebted to Gunnar Eklund, Ph.D., for valuable help with the statistical part of this study.

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Splenectomy in Childhood

A Clinical and Immunological Study of Forty-two Children Splenectomized in the Years 1951–1958

by OVE BROBERGER, FRANS GYULAI and JAN HIRSCHFELDT

The interest has since several years been attended to the question whether splenectomy will interfere with resistance to infection. King & Shumacher (13) gave in 1952 a report of 6 splenectomized patients 5 of which suffered from severe infections at some time after operation. Since then several authors have reported observations which point to an increased susceptibility to infection after splenectomy in childhood. Splenectomy has also been recorded in a table dealing with possible causes for a disposition to chronic infection in childhood by Giedion & Scheidegger (6). In addition Dameshek (3) has observed exacerbations of lupus erythematosus disseminatus within a few months after splenectomy. He gives a hypothesis according to which a removal of the spleen should abolish an inhibitory effect which this organ is supposed to exert upon the disease. Thus the spleen is supposed to produce humoral factors able to prevent a dissemination of certain processes of infectious or non infectious origin. According to Dameshek this would be analogous with the changes of the blood picture, appearance of nucleated erythrocytes, myelocytes and fragments of megakaryocytes, which will occur after splenectomy in patients suffering from hemolytic anemia. A parallel can also be drawn to some experiments in rats, where after splenectomy a Bartonella infection had manifested itself clinically. Before operation the animals were only carriers of the bacteria. (3)

By studying the literature of the last eight years we have found more or less detailed reports of altogether 460 splenectomized children (2, 7, 10, 11, 13, 16, 24, 25, 27). Fifty-six (12.1 %) of these have according to the authors been subjected to a decreased resistance to infection after operation. However, the malignancy of the infectious diseases reported has varied considerably with different authors. About three quarters of the patients had no doubts had serious infections; sepsis, purulent meningitis, and malignant pneumonias. In 27 of the cases the disease had a fatal outcome. The remaining patients on the other hand suffered only from more common infections as recurrent uncomplicated otitis or upper respiratory infections. A more careful study of the 43 cases from the literature will give the following informations: *Underlying disease:* The reason for operation was in 11 cases hereditary microspherocytoses, in 13 cases idiopathic thrombocytopenia, in 2 cases

traumatic rupture of the spleen, in 2 cases acquired hemolytic anemia, in 1 case hypoplastic anemia, in 14 cases Cooley's anemia, and in the remaining cases Morb. Gaucher, Letterer-Siwe's disease and other systemic diseases. It is evident that the patients of the last two groups due to the underlying disease will have an impaired resistance to infection and splenectomy can not be expected to modify the disease. The fairly low number of children who underwent splenectomy because of traumatic rupture of the spleen among the children with severe postoperative infections indicates that a chronic underlying disease may be of considerable importance for a decreased resistance to infection.

Age at splenectomy: There is a significant difference between infants and older children. The whole material comprises 56 (out of 460) children who were operated upon before an age of 1 year. Thirteen of these infants (22.4 %) got later severe infections and 7 of them died. After an age of 1 year, the percentage of children with severe infections is only 10.6, despite the fact that almost all cases with a grave underlying disease were to be found in this age group. It has to be pointed out that the majority of patients with repeated infections are also to be found among the infants. However, there are considerable differences between observations made by different authors. King & Shumacher (13) noted 5 infants with decreased resistance to infections out of 6 splenectomized patients, Gross (9) only 1 out of 11.

Type of infection: Twenty-one children suffered from purulent meningitis and 11 from sepsis. The remaining 24 cases were distributed among otitis (with complications), recurrent pyuria and abscesses. Results of bacterial cultivations are reported in 22 cases. In 12 cases pneumococci were grown (the cause of meningitis in 10 children), in the remaining cases meningococci, Friedländer bacilli, Escherichia coli, beta-hemolytic streptococci and Salmonella bacteria were grown.

It is evident that no definite conclusions can be drawn from such a heterogenous material, where not only variations in observation time and composition of the underlying disease but also the social environment and the actual epidemiological situation will modify the results. There is in fact only one conclusion justified to be drawn: A serious underlying disease and an age at operation below one year will enhance the risk for an impaired resistance to infection.

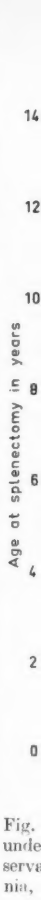
In order to find out which informations could be gained from a Swedish material we have made a follow-up study of 42 children who underwent splenectomy for different reasons in the years 1951–1958.

Material

The material comprises 42 children from the Pediatric Clinics of Karolinska Sjukhuset, Stockholm and Akademiska Sjukhuset, Uppsala. According to the underlying disease the material has been divided into three groups, one group of 11 children with *hereditary microspherocytosis*, one group of 11 children with *idiopathic thrombocytopenia* and one group of 20 children with *traumatic rupture of the spleen*. The material is graphically represented in Figures 1–2 with regard to observation time and age at operation.

Methods

A detailed sick history was recorded in each case. Special interest was put on passed diseases of infectious or non-infectious ori-



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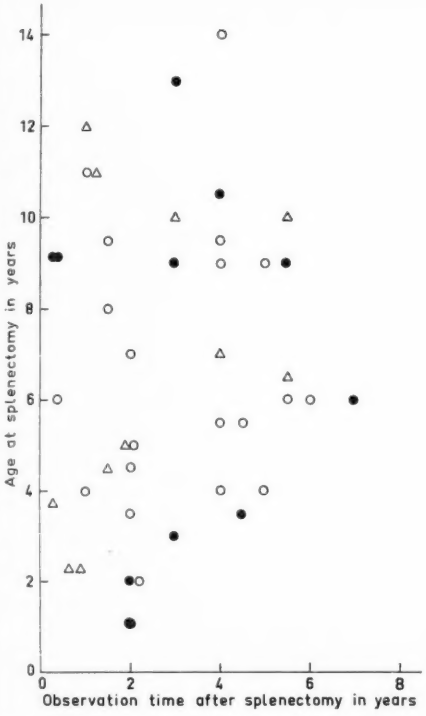

Fig. 1. Presentation of material according to underlying disease, age at operation and observation time. △ = idiopathic thrombocytopenia, ● = hereditary microspherocytosis, ○ = traumatic rupture of the spleen.

gin, physical and mental development before and after splenectomy. All patients were subjected to a thorough physical examination.

The following *laboratory investigations* were performed according to standard laboratory methods: estimation of hemoglobin, white and red cell count, differential blood count, reticulocyte and platelet count, determination of osmotic fragility and mean diameter (Price-Jones curve) of erythrocytes, serum-bilirubin and sedimentation rate. The serum-proteins were quantitatively estimated by paper-electrophoresis. Searching for LE cells was performed in each case and the urine was examined with regard to sugar, protein, cells and cylinders.

Sera from 30 of the patients were examined with regard to *bactericidal activity*. For this investigation a pure culture of coagulase negative staphylococcus albus cultivated in BHI broth was used. One tenth ml of an 18 hours old BHI-culture of staphylococcus albus, diluted to 10^{-6} was thoroughly mixed with 1.0 ml of the serum to be tested. Parallel samples of 0.1 ml were pipetted off immediately and after 10 hours incubation at 37°C and were equally distributed on BHI-agar dishes. The dishes were incubated at 37°C for 24 hours, whereafter the number of colonies was recorded. At each estimation three parallel samples of serum was examined and the mean value calculated.

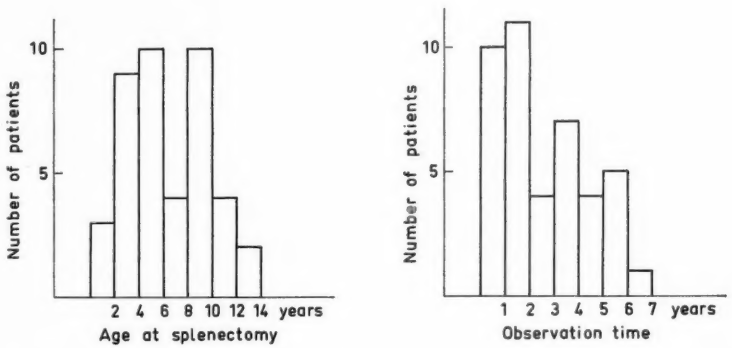

Fig. 2. Distribution of material according to age at splenectomy and observation time.

In addition sera from 28 of the patients were investigated with regard to the immune electrophoretic pattern. Immune electrophoresis was performed according to Grabar & Williams (9) in the micromodification of Scheidegger (22). This technique combines ordinary electrophoresis in agar gel with two-dimensional agar gel diffusion as developed by Ouchterlony (17). By electrophoresis in agargel, the antigens are first separated. Antiserum is then added in longitudinal basins parallel to the electrophoretic migration axis. Diffusion of the electrophoretically separated antigens against their corresponding antibodies will after a suitable time interval give rise to precipitates visible as curved opalescent lines in the gel. The peak of the precipitate, which is also the point nearest to the antibody-basin, is taken to indicate the average electrophoretic position of the antigen. After development of the immune electrophoretic pattern in a humid chamber for about 20 hours at room temperature, the microscopic slides were dried, stained with Amido Black B and photographed in transmitted light. All observations were made on photographs magnified 4.5–4.8 times. Further technical details will be published elsewhere (12). The sera to be investigated were tested against two different immune sera prepared on rabbits by immunizing with human serum. The immune electrophoretic pattern of immune serum No I, a commercial preparation from Behring Werke is seen in Fig. 4. Immune serum No 2 was kindly made available by Professor Birger Broman, Rättskemiska Institutionen, Stockholm, and proved more appropriate for demonstrating certain components in the alfa$_2$-globulin region. The sera from splenectomized patients were all kept in the deepfreeze until for use. The different components were identified by comparison with pure fractions kindly provided by AB Kabi or commercial purified antibodies from Behring Werke. In this way albumin, alfa$_2$-macroglobulin, beta$_1$-lipoprotein, transferrin and gammaglobulin were identified in the immune electrophoretic pattern. These components in our experiments on the whole agreed well with the descriptions on record. Lipoid staining made it possible to identify the alfa$_1$-lipoprotein, which also showed the same electrophoretic position and other precipitate characteristics as the alfa$_1$-lipoproteins described by others (26). The component β_2A (14) was identified by absorption of immune serum with umbilical serum which lacks this component (12). It is observed as an extended precipitate partly overlapping with the gamma-globulin precipitate. As β_2X is denoted a precipitate which for the present moment can not be correlated to any previously described components and which is very susceptible to storing effects (Fig. 4).

Results

As can be seen from Tables 1–3 severe infections were recorded in two patients, both belonging to the group of idiopathic thrombocytopenia. One of these infections was a purulent meningitis caused by hemophilus influenzae, the other an otitis with mastoiditis caused by pneumococci. It is significant that these infections occurred in two patients who did not respond well to splenectomy with regard to an improvement of the underlying disease. Thus it is impossible to determine to what extent the underlying disease did contribute to the decreased resistance to infection and how much could be ascribed to splenectomy *per se*.

Nine children had suffered from more than 1–2 common respiratory infections a year. These infections were with one exception never more prevalent than 3 a year and did not last longer than 7–10 days.

Epidemic diseases were not common. Five cases of measles, 3 cases of chickenpox and 2 cases of mumps were recorded.

TABLE 1. *Hereditary microspherocytosis.*

Age at splenectomy (years)	Sensitivity to infection before splenectomy	Observation time (years)	Complaints	Status	Upper respir. tract infection	Epidemic disease	Severe "Asiatic" influenza	Severe infections	Other infections
6	0	7	0	0	Rarely	None	0	None	0
3½	0	4½	0	0	Sometimes	Parotitis epid.	+	None	0
10½	0	4	0	0	Rarely	None	0	None	0
13	0	3	0	0	Sometimes	None	0	None	0
9	Several attacks of otitis media	3	0	0	Rarely	None	0	None	0
3	Several attacks of upper resp. infection	3	0	0	Rarely	None	0	None	0
2	Several attacks of upper resp. infection	2	0	Moderate anemia. Adenoid.	Often	None	0	None	0
9½	Otitis media, pneumonia	⅔	0	Slight hepatomegaly	Rarely	None	+	None	0
9½	Several attacks of upper resp. infection	⅔	Nervous	0	Sometimes	None	0	None	0
9	0	5½	0	0	Rarely	None	0	None	0
1¼	0	2	0	0	Rarely	None	0	None	0

TABLE 2. *Idiopathic thrombocytopenia.*

Age at splenectomy (years)	Sensitivity to infection before splenectomy	Observation time (years)	Complaints	Status	Upper respir. tract infection	Epidemic disease	Severe "Asiatic" influenza	Severe infections	Other infections
10	0	5½	0	0	Rarely	None	0	None	0
6½	Several upper resp. tract infections	5½	0	0	Rarely	None	0	None	0
7	0	4	0	lymfoma colli	Rarely	None	0	None	0
10	0	3	0	0	Rarely	Morbilli	0	None	0
5	Several upper resp. tract infections	2	0	0	Rarely	Morbilli and parotitis epid.	0	None	0
4½	0	1½	0	0	Rarely	Morbilli and varicellae.	0	None	0
4½	Several upper resp. tract infections	¼	0	thrombocytopenia	Sometimes	None	+	Purulent meningitis	0
12	0	1	0	thrombocytopenia	Sometimes	None	+	None	0
2¼	0	¾	0	thrombocytopenia	3 severe attacks	None	0	Otitis with mastoiditis	0
1	0	1	0	0	Rarely	None	0	None	0
3¼	0	¼	0	0	Rarely	None	0	None	0

TABLE 3. *Traumatic rupture of the spleen.*

No.	Age at splenectomy (years)	Sensitivity to infection before splenectomy	Observation time (years)	Complaints	Status	Upper respir. tract infection	Epidemic disease	Severe "Asiatic" influenza	Severe infections	Other inf. tis.
1	6	0	5½	0	0	Sometimes	None	+	0	
2	9	0	5	0	0	Rarely	None	+	0	
3	5½	0	4½	0	0	Rarely	None	+	0	
4	9½	0	4	0	0	Rarely	None	0	0	Strep. coec. angi.
5	9	Several upper resp. infections	4	0	0	Rarely	None	0	0	
6	14	Several upper resp. infections	4	0	0	Rarely	None	0	0	
7	4	0	4	Slight abdominal pains	0	Rarely	Morbilli	0	0	
8	5½	0	4	0	0	Rarely	Morbilli	0	0	0 sim.
9	7	0	2	Often tired	Normochromic anemia	Sometimes	Varicellae	+	0	
10	3½	0	2	0	0	Rarely	None	0	0	
11	8	Uncomplic. otitis med., twice	1½	0	0	Sometimes	None	0	0	
12	9½	0	1½	0	0	Rarely	None	0	0	
13	11	0	1	0	0	Sometimes	None	0	0	
14	6	0	¼	0	0	Rarely	None	0	0	
15	4	0	5	0	0	Rarely	Varicellae	+	0	
16	4½	3 attacks of urinary infections	2	0	0	Rarely	None	0	0	
17	2	0	2	0	0	Rarely	None	+	0	
18	6	0	6	0	0	Rarely	None	0	0	
19	4	0	1	0	0	Rarely	None	0	0	
20	5	0	2	0	0	Rarely	None	0	0	

Noteworthy is that the so called "Asiatic influenza" which caused a severe epidemic in 1957 did not affect more than 10 of the children. Nine children with a massive exposure to this disease (several family members affected) did not show any clinical signs of the disease.

The *physical* and *mental development* was normal in all children.

The *laboratory investigations* did not reveal anything abnormal which could not be expected with regard to the underlying disease. However, one patient who was operated upon because of traumatic rupture of the spleen had several years after operation a persistent normochromic anemia. A positive LE cell phenomenon could not be demonstrated in any case and no patient has showed any other symptoms of Lupus erythematosus disseminatus.

Thirty sera were investigated with regard to *bactericidal activity*. As can be seen from the diagram in Fig. 3 the sera from the splenectomized children do not differ from the sera from the 7 healthy control children with regard to bactericidal activity. The number of colonies varied between 0 and 9×10^1. From the work of

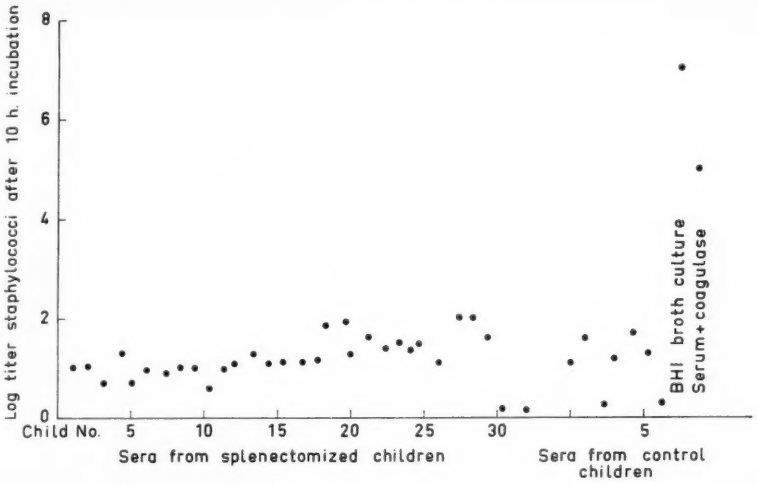

Fig. 3. Antibacterial activity (against Staphylococcus albus) of sera from splenectomized children and normal children. Vertical axis represents log. number of colonies.

Ekstedt *et al.* (5) it is proven that the number of colonies will increase up to 10^5–10^6/0.1 ml serum if the bactericidal activity is inhibited by adding coagulase. Thus we can say that the anti-bacterial activity against staphylococcus albus has not been significantly altered in the sera investigated. (These sera included sera from those two patients who showed a decreased resistance to infection after operation.)

In the present study 28 sera from splenectomized children aged 3–16 years were investigated with *immune electrophoresis*. Nine of these belonged to the group with hereditary microspherocytosis, 11 to the group with idiopathic thrombocytopenia and 7 to the group with traumatic rupture of the spleen. The immune electrophoretic pattern on the whole showed good agreement with the pattern obtained when normal sera are investigated. In some instances, however, minor differences in certain sera were noted.

In two of the sera from patients with idiopathic thrombocytopenia the alfa$_1$-lipoprotein showed a retarded migration rate. This retarded migration rate of the alfa$_1$-lipoprotein is seen in Fig. 4. Variations of the position of the alfa$_1$-lipoprotein have been found in sera subjected to aging and after pretreatment with certain enzymes (26). In those instances, however, an accelerated migration rate has been observed.

Nine of the 28 sera presented evidence of a decreased transferrin content as judged by the immune electrophoretic pattern. This component will normally give rise to an "antigen excess pattern" (Fig. 5). Only in 3 of these sera the amount was sufficient to allow a quantitative determination of transferrin according to Ramsay (18) (kindly carried out by Dr.

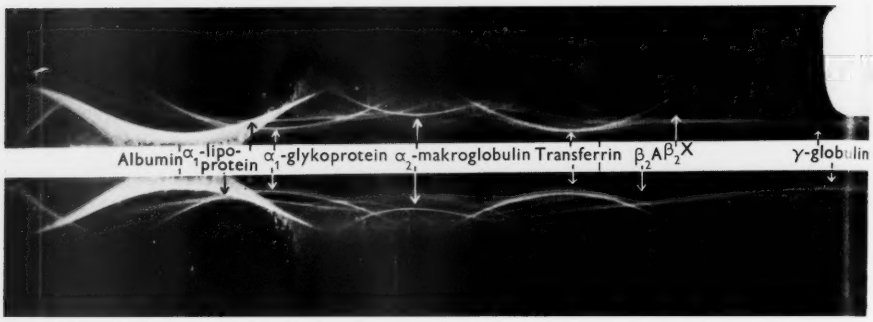

Fig. 4. Immune electrophoretic pattern of two splenectomized patients (I.F. and G.J.) against anti-human serum No I. Note the retarded migration rate of the alfa$_1$-lipoprotein from pt. I.F. (upper half) β_2A is absent in the serum from pt.IF. but can be clearly seen in the pattern from pt.G.J.

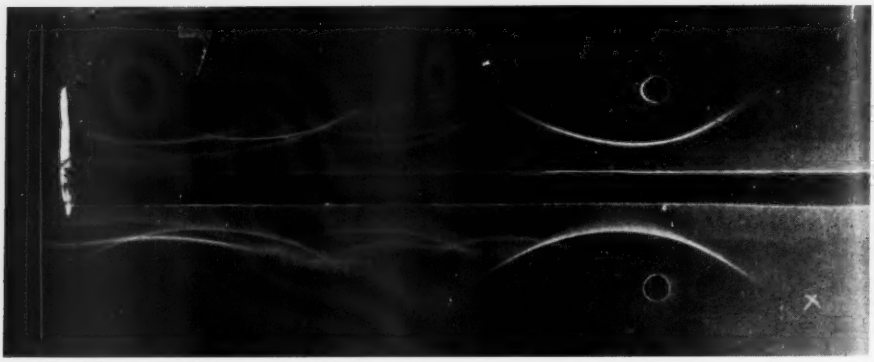

Fig. 5. Immune electrophoretic pattern of serum from a splenectomized patient (with traumatic rupture of the spleen) (upper part) as compared with serum from a normal individual (lower part). The transferrin precipitate in both sera is localized between the starting points (round basins) and the longitudinal basin. The antigen excess pattern of the transferrin precipitate visualized as a shadiness of the peak of the precipitate is absent in the serum from the splenectomized patient.

Bertil Nosslin, Kemiska Centrallaboratoriet, Malmö Allmänna Sjukhus, Malmö). These sera showed transferrin values between 208 and 215 μg %, which is well below the normal level. A simultaneous transferrin determination of sera from 4 normal children of the same age showed values between 328 and 344 μg %. It has to be pointed out that 5 of the sera (including those in which transferrin was quantitatively determined) with a decreased transferrin content as judged by the immune electrophoretic pattern came from 5 of the 8 patients with traumatic rupture of the spleen.

In 12 of the sera investigated the component β_2A was difficult to observe, being to a great extent confluencing with the gammaglobulin precipitate. This might imply that in these sera this component

is diminished or absent. In 16 sera β_2A could clearly be seen, having a position somewhat above the gamma-globulin precipitate (Fig. 4). Previous investigations of sera from splenectomized children by Scheidegger & Martin du Pan (23) have demonstrated absence of β_2A.

The gamma-globulin precipitate had an evidently normal appearance in all sera investigated, which is in good agreement with the quantitative electrophoretic estimation where no abnormalities regarding the content of different serum globulins were found.

Discussion

The anamnestic data have revealed a tendency to severe infections only in two children suffering from idiopathic thrombocytopenia, but as these patients have not responded well to operation with regard to the underlying disease, this tendency can not with certainty be ascribed to splenectomy *per se*. This is in good agreement with the material from the literature as our material only comprises children above one year of age.

In this context it would be relevant to discuss the role of the spleen in antibody synthesis. The endogenous production of antibodies is absent or very low at birth. The production starts successively during the first three months of age but full capacity may not be reached before an age of about two years. Thus the endogenous production may be suboptimal during the first year of life and it is reasonable to assume that removal of an organ involved in antibody production at this period will give rise to a greater if even only transient disturbance in antibody synthesis than later in life. However, experimental evidence for this assumption is so far lacking. A quantitative difference with regard to gamma-globulin content between sera from splenectomized children above one year of age and normal children has not been found in this investigation. Smith *et al.* (24) and Gofstein *et al.* (17) have investigated the gamma-globulin contents of sera from splenectomized patients with severe infections in the postoperative period and have not found any abnormalities. The response to antigenic stimulation has mostly been studied in adults. Myerson *et al.* (15) found no differences of the antibody titers against tetanus-toxoid between splenectomized and normal adults. The same results were obtained by Saslow *et al.* (20) when they studied the response to tularemia vaccine in 105 splenectomized patients. However, there may be some differences if the antibody response is studied in the early postoperative period. Thus Gohar (8) found lower anti-body titers against typhus antigen when injected 16 than 30 days after operation. When injected 30 days after operation the response in splenectomized patients showed no difference as compared to that of normal controls. This might imply that the role of the spleen in antibody synthesis is of only minor importance and that a loss of this organ is very quickly compensated for by other functional units of the anti-body producing system. However, the problem may be more complicated than so. The strength of the response may even be determined by the way of injection and the antigen used. Rowley (19) found practically no titers after intravenous injection of sheep erythrocytes in 13 out of 14 splenectomized human

beings, while all controls showed considerably high titers.

Other factors than specific anti-bodies are involved in resistance to infection. However, very little is known about this so called unspecific resistance. It is a well-known fact that infants suffering from the hereditary form of agammaglobulinemia are to a certain degree resistant to some virus and gram-negative bacteria. Phagocytosis is known to operate in this field but accurate methods for a quantitative estimation are not available. Properdin may play an important role but clinical evidence for this is to a great extent still lacking. Recently Saslaw & Carlisle (21) have published an investigation of properdin titers in 105 splenectomized patients. They found significantly low titers as compared to those of 113 normal controls. Thirty of our sera have been investigated for bactericidal effect in vitro. This effect may to a great extent depend upon factors involved in unspecific resistance. However, an effect of specific antibodies can not be ruled out by the method of investigation. (Complement has not been inactivated as C_3 is necessary for the action of properdin.) The investigation did not reveal any abnormalities which is in good agreement with the clinical observations.

The immune electrophoretic investigation of 28 sera from splenectomized children showed minor differences regarding the components alfa$_1$-lipoprotein and β_2A. The significance of these findings can not be clinically evaluated, however, as very little is known about the physiological function of these components. More interesting is the finding of a decreased transferrin content of some of the sera. It is surprising that the majority of these sera belonged to the small group of patients (investigated with immune electrophoresis) with traumatic rupture of the spleen. Only one of these patients suffered from a moderate normochromic anemia.

Summary

A clinical and immunological follow-up study was performed in 42 children who were splenectomized for three different reasons (hereditary microspherocytosis, idiopathic thrombocytopenia or traumatic rupture of the spleen) in the years 1951–1958. All children except one was in the age group 2 years or older. The investigation gave no evidence of an impaired resistance to infection as a result of splenectomy *per se* in the material studied. Though two patients suffering from idiopathic thrombocytopenia showed a tendency to severe infections this tendency could not with certainty be ascribed to the splenectomy as these patients did not respond well to the operation with regard to the underlying disease. The bactericidal activity of sera from 30 of the children was investigated but showed no differences as compared to that of sera from normal children. Sera from 28 of the children were studied with immune electrophoresis. Minor differences as compared with the immunelectrophoretic pattern of normal sera were noted regarding the components alfa$_1$ lipoprotein and β_2A. In addition 9 of the sera showed a decreased transferrin content as judged by the immune electrophoretic pattern. Five of these sera came from children who had been splenectomized because of a traumatic rupture of the spleen.

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Metachromatic Leucodystrophy—a Generalized Lipidosis

Determination of Sulfatides in Urine, Blood Plasma and Cerebrospinal Fluid

by BENGT HAGBERG and LARS SVENNERHOLM

Late infantile metachromatic leucodystrophy (Mb Greenfield) is suitably classified as a generalized lipidosis due to the accumulation in various organs of lipid metachromatic substances (3, 5, 7, 9). In 1958 these substances were shown to be sulfatides (sulfuric acid esters of cerebrosides) in investigations made independently in three different parts of the world (3, 7, 9). Large amounts are found not only in the nervous system but also in the parenchymal liver cells, the mucous folds of the gall bladder wall and the kidney tubules. The disease is histologically and chemically well defined and the clinical picture is characteristic (8). According to Austin the clinical diagnosis can be verified during life with simple urinary tests (1, 2).

This report deals with the chemical changes in urine, blood plasma and cerebrospinal fluid of three cases with a typical clinical picture which were diagnosed during life.

Material

Normal material comprising 120 healthy infants, children and adults was investigated with urinary tests. Blood plasma was chemically analysed in six normal children. No investigations on the cerebrospinal fluid (CSF) were made in this normal group. Three cases with metachromatic leucodystrophy were examined and their clinical histories are briefly reported below.

Case 1. A.L., a girl 3¾ years of age, the only child in a healthy family. She learnt to walk when 15 months old but never walked well. From about this time successive development of a clinical picture very similar to the syndrome of Guillain-Barré with hypotonic tetraplegia, absent deep reflexes, severe pain in legs and arms. However, atactic symptoms, bilateral positive Babinski and a deterioration of her speech soon appeared in addition. When three years old she rapidly deteriorated and the tetraplegia changed from a hypotonic to a hypertonic type. For the last half year of life she was in a state of decerebrated rigidity, blind with total optic atrophy and severely mentally retarded. A cholecystography was performed with a nearly normal result when she was 2¾ years old. One year later no filling of the gall bladder could be achieved. Autopsy with microscopic and chemical examination verified the diagnosis (Hagberg *et al.*, to be published).

Case 2. I.M., a girl, 5 years of age. Her elder sister died from the same disease when 6 years old, the diagnosis being verified by microscopic and chemical examination at autopsy. I.M. developed normally until

nearly three years of age. From about that time her gait was observed to be unsteady and weak. When four years old she had a severe epileptic fit. At that time she could still walk, had a slight hypertonic paraplegia with positive bilateral Babinski, intention tremor in her hands and a slight dysarthria. No nystagmus or optic atrophy. At cholecystography no filling of the gall bladder could be obtained. Now 5 years old she is in a bad condition, neither able to walk, nor to sit without support and has bulbar symptoms.

Case 3. R.A., a boy, 4 years of age in a healthy family. Progressive hypotonic tetraplegia started insidiously between one to two years of age. Positive bilateral Babinski was an early finding. Atactic symptoms and mental deterioration were soon also observed. At 2½ years of age he was highly demented with hypertonic fits and periods with cerebral fever. A clinical picture of decerebrate rigidity progressively developed. Other signs were weak or absent deep reflexes, nystagmus, and left optic atrophy with no pupillary reactions to light. Cholecystography did not reveal any functioning gall bladder. During the last year he has been in a more stationary stage. The diagnosis was verified by histological examination of a cutaneous nerve (13) from biopsy material (P. Sourander, personal communication).

Methods

Urine. The sediment was examined microscopically according to Austin (1). The total lipids in the urinary sediment were extracted with chloroform-methanol (2:1, v/v) and the lipid soluble metachromatic substances were estimated by a semiquantitative paper chromatographic procedure (Hagberg & Svennerholm, to be published). The lipid extract was dialysed against water to remove water soluble contaminants. After dialysis an aliquot of the lipid extract was chromatographed on Schleicher & Schüll 2043b papers with tetrahydrofuran-diisobutylketone-water 45:5:5 (v/v) as solvent. The amount of lipid extract applied to the paper was equivalent to 2 mg of urinary creatinine. The sulfatides were visualised by cresyl violet and Rhodamine B. The amount of sulfatides was estimated by comparing the size and intensity of the metachromatic spots of urine with those of known amounts of brain sulfatides.

Blood

The total amount of cerebrosides in plasma was determined according to Svennerholm & Svennerholm (12). The sulfatides were analysed by the same paper chromatographic technique as used for urine.

Cerebrospinal fluid

Total protein was determined according to Goa (6) with the biuret method. The CSF proteins were concentrated before electrophoresis by high pressure ultrafiltration. Paper electrophoresis was performed according to Mellander *et al.* (11).

Results

Urine

Microscopical examination. Large metachromatic granular bodies of the type described by Austin (1) were only found in the three cases with metachromatic leucodystrophy.

Chemical analysis. The results are given in Table 1.

Blood

The total amounts of cerebrosides in plasma were for the three clinical cases A.L. 3.9, I.M. 3.4 and R.A. 3.7 and for the six normals 3.84 (range 2.7–4.7) mg/100 ml. The sulfatides were only analysed semiquantitatively. No significant differences were found between the patients and the normals.

Cerebrospinal fluid

The concentration of total proteins was markedly increased in our three cases but

TABLE 1. *The concentration of sulfatides in urinary sediments of normals and three cases of metachromatic leucodystrophy.*

(All figures are expressed as the amount of sulfatides per 2 mg creatinine.)

	Content of sulfatides in μg					
Age	< 0.5	0.5–2.5	2.6–10	11–25	26–100	> 100
0–2 months			3	9	3	2
2–4 ,,			5	6	6	2
4–6 ,,			1	3	1	1
6–8 ,,	1		3	4	2	
8–12 ,,			14	4	1	
1–2 years		2	6	3		
2–4 ,,		5	4	A.L., R.A.	A.L.	
4–8 ,,		1	1		I.M.	
8–15 ,,			2			
> 15 ,,	5	16	4			

TABLE 2. *The total protein concentration in cerebrospinal fluid and its relative distribution by zone electrophoresis on filter paper.*

	Case A. L.[a]	Case I. M.	Case R. A.
Total protein mg/100 ml	145	115	140
Albumin in per cent	68.5	66.3	73.1
α_1-globulin ,,	6.0	5.8	6.1
α_2-globulin ,,	8.8	7.5	8.5
β-globulin ,,	10.2	5.1	11.5
γ-globulin ,,	7.1	8.5	6.6

[a] Agar electrophoresis, 9 months later (performed by Dr. A. Lowenthal, Antwerp). Total protein 167 mg/100 ml. Albumin 64.7 %, α_1 6.1 %, α_{2_1} 8.1 %, α_2 1.4 %, β_1 9.5 %, β_2 3.0 %, γ_1 2.7 %, γ_2 4.5 %.

the paper electrophoresis showed a normal relative distribution as can be seen in Table 2. Sulfatides were present in CSF of the three clinical patients but due to the small amounts it is impossible to evaluate if there was more than in normals.

Discussion

Austin (2) concluded that a positive test for metachromatic lipids of urinary sediments (sulfatides) is pathognomic for late infantile metachromatic leucodystrophy. Contrary to Austin we found that sulfatides are excreted in the urines of all the normal persons (infants, children and adults). Healthy infants less than one year of age, excreted as large, or even greater amounts than our three patients. However, these patients 3–5 years old, had much more sulfatides in their urines than normal children of the same age. Large amounts of urinary sulfatides were also found in children with other wide spread demyelinating diseases (Hagberg and Svennerholm, to be published). Thus, diagnostic conclusions must be drawn very cautiously, especially as this test is further limited by large variations in the daily excretions of sulfatides, making all quantitative estimations rough.

In addition to a distinct strong sulfatide spot there was in the paper chromatograms of urinary sediments from our three cases with proved metachromatic leucodystrophy a second intense metachromatic spot with a somewhat lower R_F-value. This second spot has only been seen in about 10 per cent of lipid extracts from the urinary sediments of normals and was never so intense as in the lipid extracts of the metachromatic cases. In our experience the ratio sulfatides to total lipids is also much larger in late infantile metachromatic leucodystrophy than in normals or patients with other demyelinating diseases. While Austin's analyses of lipids without chromatography (2) only imply a visualization of microscopically already verified metachromatic substances, a differentiated chromatographic pattern is

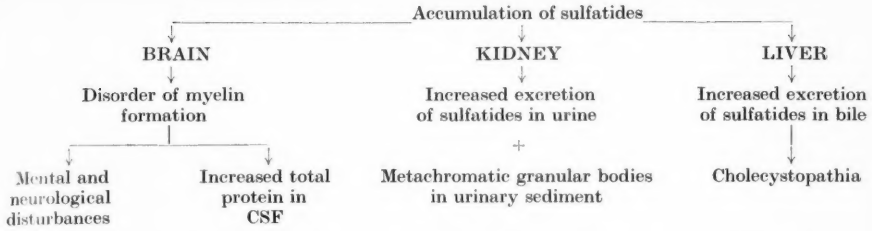

Fig. 1. The clinical manifestations of the disturbed sulfatide metabolism in late infantile metachromatic leucodystrophy.

obtained with the method used here. But the most reliable laboratory test up to now seems to be the microscopical examination. Large metachromatic granular bodies in toluidine blue stained sediments have only been found in patients with metachromatic leucodystrophy.

The increased content of protein in the CSF of our three patients as well as in earlier cases described (5, 8, 10) is interesting. To our knowledge electrophoretic separation of the protein fractions has not been performed earlier in this disease. The increased protein content combined with a normal distribution of the electrophoretic components is surprising but may at least to some extent be explained by the effect of a non specific polyradicular irritation, as metachromatic substances have also been found in damaged nerve roots (8). In good agreement with these findings is the clinical picture of the earlier stages, which is often very similar to the syndrome of Guillain-Barré.

Another interesting fact in relation to the disturbed lipid metabolism is the non-functioning gall bladder in our three cases. In an earlier report it was shown at autopsy (8) that the gall bladder walls in two cases were distended with macrophages containing abundant metachromatic and PAS-positive material. These findings may be plausibly explained by an increased excretion of sulfatides in the bile, causing secondarily a gradually increased accumulation of sulfatides in the wall of the gall bladder. The final result is a progressive loss of the concentration ability of the gall bladder, which also could be followed at repeated X-ray examination in Case 1. Whether a pathological X-ray finding is a constant sign in the later stages of the disease cannot be assessed, as X-ray examinations have not been reported previously.

The effect of the increased synthesis of sulfatides on the human organism is summarized schematically in Fig. 1.

Summary

Three cases of late infantile metachromatic leucodystrophy, diagnosed during life, have been investigated with microscopic studies of urine and with chemical analyses of urine, blood plasma and cerebrospinal fluid. Comparable studies have been made on corresponding materials from healthy infants, children and adults.

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Autopsy Findings in Three Cases of Hypsarhythmia (Infantile Spasms with Mental Retardation)[1]

by CHARLES M. POSER and NIELS L. LOW

The combination of infantile spasms with mental deterioration of a progressive nature has been previously described by several authors. The literature concerning this clinical entity was recently reviewed by Low, Bosma, Armstrong, and Madsen (9). New experience in the treatment of children affected with this syndrome has also been the subject of recent publications by Sorel (17) and by Low (8). Most of these children have been found to exhibit a peculiar electroencephalographic abnormality originally described in 1952 by Gibbs and Gibbs in their *Atlas of Electroencephalography* and further characterized by Gibbs, Fleming and Gibbs (6), and named hypsarhythmia. To our knowledge, no report of the pathologic findings in the brains of children dying with this condition has been published.

Three patients who were observed clinically by one of us (NLL) and have been previously included in a review of the clinical picture of this disease (9) are the subjects of this post-mortem report.

CASE 1: *D. H.* (*Case 8, (9)*)

Clinical History. This full-term bottle fed boy was hospitalized at age six weeks because of irritability, loose stools, and fever. General physical, developmental, and neurological examination at that time revealed normal findings with normal skull x-rays, normal EEG and spinal fluid. Shortly before he was three months old, he started typical infantile spasms. These seizures occurred very many times daily for the rest of his life. Repeat examinations revealed a marked regression of this baby's developmental milestones and at five and one-half months he first had swallowing difficulties. At that time he no longer followed a moving light or other objects; he had no head control anymore; the neck musculature was quite limp, the extremities had an increased tone, and the deep tendon reflexes were hyperactive. The child died following aspiration of milk at seven months of age.

[1] Read in part at the Scientific Session of the Brain Research Foundation in Chicago, 31 March 1955.

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Pathologic Findings

The post-mortem examination was performed 16 hours after death. The significant findings consisted of acute pulmonary emphysema and pneumonia involving both lower lobes, and an acute obstructive type of bronchitis. The terminal event was felt to be an acute broncho-pneumonia.

The brain weighed 750 g and was felt to be firmer in consistency than usual. On cut sections it was said to show extensive areas of demyelinization, especially in the cerebral hemispheres. No other abnormalities were noted. (Gross findings by courtesy of Drs. Hicks and Bennett, Ogden, Utah.)

Histologic Examination

Fragments of the brain were available for examinations. They had been fixed in formalin and were examined in frozen, paraffin and celloidin sections by means of staining with Oil Red O, Nile Blue Sulfate, Congo Red, PAS, thionine, hematoxylin eosin, Woelcke myelin sheath method, Bielschowsky, Holzer, and Nissl methods.

There were extensive areas of spongy degeneration which involved almost exclusively the deeper layers of the cortex in all areas examined, but most pronounced in the frontal lobes. This spongy degeneration resulted in a complete degeneration of neurons and vacuolization of the cerebral ground tissue. There was evidence of considerable capillary proliferation with secondary endothelial degeneration. Throughout the areas of spongy degeneration there was a striking absence of glial response, of phagocytes, and of products of degeneration. The latter were found only in rare, scattered areas as intra-adventitial masses of brilliant red staining material with the Oil Red O stain. PAS, Congo Red and Nile Blue Sulfate methods all gave negative results. No signs of an inflammatory response could be seen in the central nervous system: the glial response was, as stated, minimal, there were no perivascular cellular collections, the meninges were free of cellular infiltrates. The neurons of the cortical layers not involved by the spongy degeneration showed scattered, relatively mild changes such as early chromatolysis and loss of Nissl substance. In a few instances hyalinization and complete disappearance of the cell body occurred. Neuronophagia was conspicuously absent. These changes occurred in scattered areas of the cerebral cortex, showing the changes of early, acute necrosis. The subcortical white matter showed only mild changes consisting of what might be called chronic low-grade edema and probably represents a milder degree of the process involving the grey matter. Nonetheless, the difference in the type of involvement was quite striking. Here again, there was absence of glial, phagocytic or inflammatory response. Myelin sheath stains, however, revealed a diffuse, irregular, moth-eaten type of demyelinization which was most severe in the vicinity of the areas of cortical spongy degeneration, most marked in the frontal lobes (Fig. 1). Neither in the grey nor in the white matter could giant, naked nuclei of

Fig. 1. Case 1: Occipital lobe. Diffuse but selective demyelination of the subcortical fibers (Woelcke).

Fig. 2. Case 1. Junction of internal capsule and putamen. Spongy degeneration and edema of both grey and white matter. Relatively mild glial reaction (Woelcke).

the Alzheimer type II glia be found. No inclusion bodies were seen in any of the numerous sections that were examined for that purpose.

The areas of the central nervous system affected by the spongy degeneration failed to follow any systematic distribution. Almost all grey masses of the brain and spinal cord were involved, but in a very irregular manner. The basal ganglia (Fig. 2), especially the caudate nuclei, were affected but the amygdala and the hippocampus were not strikingly involved. The brain stem was also involved, again in the nuclear masses, although to a lesser extent than the more cephalad structures. The anterior horns of the spinal cord showed rare scattered areas of mild spongy degeneration. The cerebellum was relatively spared; the Purkinje cells and the granular layers appeared intact while the cerebellar white matter was affected by mild edema. Mild vascular dilation and perivascular edema without vascular engorgement were found throughout the areas of grey matter involved by the milder degrees of chronic edema.

In summary, the basic pathologic process appears to be a chronic edema of the grey matter, most pronounced in parts of the cerebral cortex, going on to spongy degeneration, with relatively mild involvement of the white matter and mild, probably secondary demyelinization. There was a striking absence of glial and phagocytic response as well as of "Abbau" products even in the most severely involved areas. The scattered, acute neuronal changes are felt to be the result of cerebral anoxia concomitant with the terminal illness.

Case 2. (*Case 9*, (*9*))

Clinical History: This baby was born in breech presentation, she had one convulsion two or three days after birth, showed some cyanosis during the first few days, and a few recurring convulsions during the first four months. At 12 days of age, phenobarbital was given and the dosage was reduced by the mother shortly before the patient was four months old. Forty-eight hours later, status epilepticus ensued and 15 long major seizures occurred in less than 24 hours. When the patient was admitted to the hospital typical infantile spasms were observed which had not been recognized by the parents as being significant. The child was limp and less responsive than normal babies at age four months. Subdural taps and roentgenograms of the skull revealed no abnormality. Electroencephalogram showed multiple seizure foci. At eight months of age the normal motor, vocal, behavioral and adaptive functions expected in a 6-week-old infant were not present. Convulsions recurred occasionally and infantile spasms occurred many times daily. When the child was one year old, she started having increasing difficulty in swallowing and, at the same time, became thin and hypertonic, although she had been fat and limp earlier. During the last three months of life she had to be fed by gastric tube because of lack of swallowing ability.

Fig. 3. Case 2. Section through the corpus callosum and the head of the caudate nucleus. Diffuse, moth-eaten demyelination (Woelcke).

She died at 20 months of age, emaciated and with severe spastic quadriplegia with fixed deformities. Frequent seizures continued to recur until the last day.

Pathologic Findings: There were no abnormal findings outside of the central nervous system. The brain weighed 810 g in fixed condition; it appeared normal except for a mild degree of cortical atrophy of the frontal lobes and a mild, generalized ventricular dilatation, especially marked around the third ventricle and aqueduct of Sylvius.

Histologic Examination: The same techniques were used as in Case 1.

The entire central nervous system revealed a mild, diffuse, irregular demyelinization involving, to varied degrees, all areas of white matter (Fig. 3). This demyelinization appeared to be secondary to a widespread, generalized, chronic edema of the white matter, which in some areas took on the appearance of spongy degeneration. Nowhere was this spongy degeneration so advanced as in Case 1. Strikingly, in this case, the pathologic process involved primarily the white matter with relative sparing of the grey. Here, as opposed to Case 1, the white matter stood sharply against adjacent, intact grey matter. In addition, a moderately severe microglial response was present throughout the central nervous system, involving all areas of white matter and spilling over into the adjacent cortex. Rare glial nodules and perivascular cuffs were present although the meninges showed no such signs of an inflammatory response (Figs. 4). Congo Red, PAS and Nile Blue sulfate stains gave negative results. Oil Red O stain revealed rare, very small accumulations of brilliant red material in the wall of small blood vessels. There was a striking absence of phagocytes throughout the areas of destruction.

The cerebral cortex showed small, scattered areas of early necrosis with chromatolytic neuronal changes, but no neuronophagia was seen. The grey masses of the basal ganglia were not as well preserved as those of the cortex since areas of early spongy degeneration were found in the globus pallidus and putamen.

The spongy degeneration involved the entire centrum ovale of the cerebral hemispheres but in an irregular pattern the most peripheral areas, i.e., the axes of the convolutions

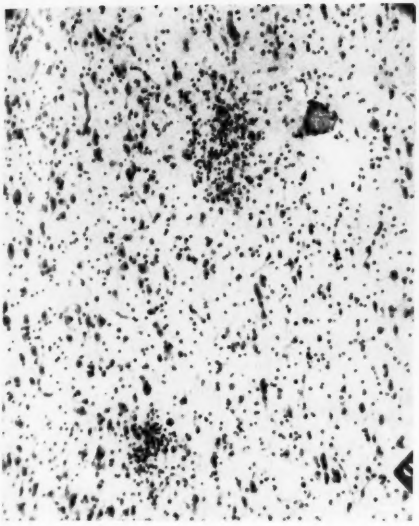

Fig. 4. Case 2. Claustrum. Diffuse glial reaction and two glial nodules (Nissl).

Fig. 6. Case 2. Section through the hippocampus. Selective spongy degeneration of the rubrothalamic fibers, the optic tract and the tapetum. Mild diffuse demyelination of the subcortical white matter (Woelcke).

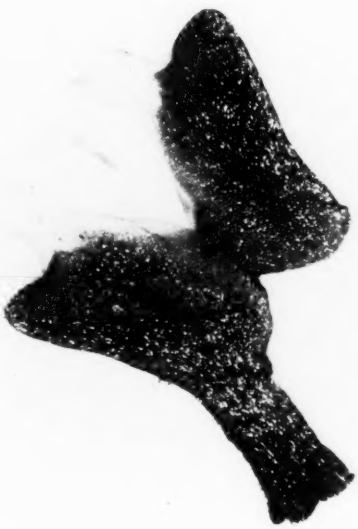

Fig. 5.

especially in the occipital lobes, in the insular cortex and the anterior part of the temporal lobes. The involvement, however, was not symmetrical. The optic chiasm was the most severely involved of all areas of white matter while the spinal cord was essentially spared (Fig. 5). The white matter was most affected immediately adjacent to the cortex so that in the occipital lobe one might get the impression that there was an almost selective involvement of the U-fibers. In some areas, such as the walls of the lateral ventricles, the spongy state showed signs of early necrosis. As expected, the lateral areas of the pons and medulla were more severely affected than the medial ones. The cerebellum was also diseased; there was a scattered loss of Purkinje cells and rarefaction of the granular layer. An example of the irregular manner of involvement of the white matter is the fact that the corpus callosum appeared intact although the tapetum, the capsule of the red nucleus and the rubro-

thalamic fibers are severely affected (Fig. 6). Thus, it would appear that the involvement is non-systemic but in some ways selective. In summary, the spongy degeneration is again present in this case but predominantly involving areas of white matter with relative sparing of the grey matter. There is a moderate glial response, which in some areas resembles a definite inflammatory reaction. Again, however, there is a striking absence of phagocytosis and products of degeneration. Some acute changes are present in the cortical grey matter which may represent anoxic effects resulting from the terminal illness.

CASE 3. (*Case 10*, (*9*))

Clinical History: This girl started having seizures at ten hours of age. They occurred many times daily and consisted of sudden, brief, jerky movements of the extremities and some facial twitching. At age three weeks she weighed 3765 g. General examination was normal and the baby had good Moro and tonic neck reflexes. There were normal movements of the tongue, palate, and eyes, and the general muscle tone appeared normal for the age. The fundi also were normal, the deep tendon reflexes were symmetrical, and she had bilateral Babinski signs.

Administration of phenobarbital, Mebaral, Mysoline, and Dilantin did not modify the number or severity of the seizures. Whenever ill with respiratory infection or having loose stools, some brief generalized convulsions occurred. Until 18 months of age, there were minor motor seizures, which could be best described as infantile spasms, during almost every hour. After that age, the seizures diminished considerably in frequency. At 22 months of age, development in all spheres was that of a newborn baby. The extremities were markedly hypotonic during the first year but gradually developed increased tone. At this time, she was severely spastic, with fixed deformities and hypertrichosis of the abdomen, pubic area and thighs. At 12 months of age she lost the gag reflex and had increasing difficulty with swallowing which progressed until the second birthday, after which she could be fed only by nasogastric tube. In the meantime the fundi had become very pale. She died in complete decerebrate rigidity at age 25 months.

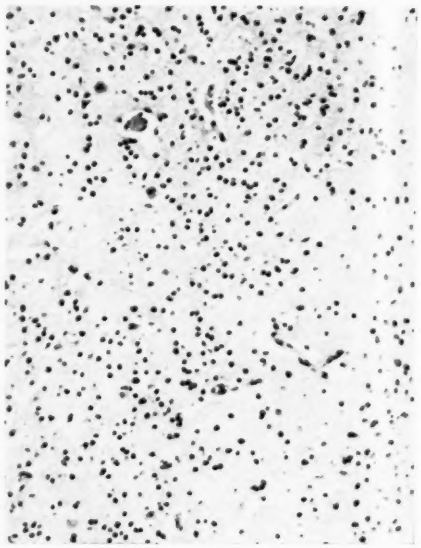

Fig. 7. Case 3. Temporal lobe. Subcortical white matter. Diffuse, early spongy degeneration. Moderate glial reaction (H & E).

Pathologic Findings: The only abnormal finding at the general autopsy was a congenital hour-glass deformity of the esophagus. The fresh brain weighed 850 g and showed only a mild degree of atrophy of the paracentral lobules bilaterally. There was no significant ventricular dilatation.

Histologic Findings: The basic pathologic process was, as in the other two cases, a state of chronic edema which in some areas went on to spongy degeneration and necrosis (Fig. 7) As in the first case, and as opposed to the second one, in this brain the process was primarily involving the grey matter. In the cortex, however, instead of the deep layers of the cortex being involved,

it was the middle layers which were affected. The spongy state was less well organized and many more areas of frank necrosis were present. A moderate glial response was present although no frank nodules, perivascular cuffs or meningeal infiltrates could be found. Even though the areas of spongy degeneration were accompanied by severe cell necrosis and loss, no neuronophagia and no phagocytic responses were observed. Oil Red O, PAS, Nile Blue sulfate and Congo Red stains revealed only rare, minimal adventitial collections of neutral fat material. The involvement was irregular, scattered and non-systemic. It involved only very, few areas caudal to the basal ganglia, sparing the spinal cord. The cerebellum showed somewhat more severe involvement than did the previous two cases with small necrotic foci involving Purkinje cells and granular layer. On the other hand, the cerebellar white matter showed a moderate degree of spongy degeneration. The occipital cortex was the most severely affected. The white matter showed a very mild, diffuse, moth-eaten type of demyelinization, as well as the generalized, moderate gliosis mentioned above. In the brain stem, the external capsule and the cerebellum, as opposed to the subcortical white matter, there was selective involvement of the white fibers as opposed to the grey matter. This is a picture which strongly resembles the findings in Case 2, while the cerebral hemispheres present a picture more similar to Case 1. In summary, the chronic edema and spongy degeneration found in the previous two cases again appeared to be the basic pathological process. While in the cerebral cortex and basal ganglia the grey matter was most severely involved, in the brain stem the white fibers appeared almost selectively involved. Moderate gliosis but absence of phagocytic response and "Alzbau" products are again a major finding. In this case the process appears to be somewhat more acute as judged from the more frequent occurrence of areas of frank necrosis throughout the central nervous system.

Discussion

In a previous communication (9) from the University of Utah, the clinical syndrome of infantile spasms was presented and discussed in detail. It was pointed out that most of the children so affected were normal during the first weeks and months of life but they started deteriorating coincidentally with the onset of seizures. The striking similarity of children with phenylketonuria, a metabolic disease, to children with infantile spasms was emphasized in the earlier paper. The similarity in clinical progression, in seizures and EEG (5), presents the hypothesis that many, not necessarily all, infants with this syndrome may be affected with a disease of chemical origin. There may be several types of enzymatic deficiencies that can manifest themselves in one single syndrome, only one of which has been separated from the group so far, namely phenylketonuria.

Most children with infantile spasms and hypsarhythmia survive beyond the first few years; in fact, they do not deteriorate beyond a certain level which they reach sometime between two and three years of age, and the seizure and EEG picture changes gradually after that age. If the hypothesis is correct that most of the children so affected belong to specific metabolic entities, they should have similar pathological changes in the central nervous system. Only the most severely affected of these infants die while the deterioration is active; the less acute cases will not come to autopsy unless the children die due to unrelated causes. Therefore, the findings that are presented here must be active and extreme ones compared to the children who show signs and symptoms

later and survive with less severe mental retardation.

The three cases presented in this report have in common a similarity in pathological findings in addition to their parallel clinical course.

The three brains we were able to study show the same basic pathologic process characterized by chronic edema of varying degree of intensity, resulting in a spongy degeneration. In the first case, it is the grey matter that is primarily involved; in the second, the white matter with remarkable sparing of the grey; while in the third, both the grey and the white matter are affected, albeit the first more severely. Demyelinization certainly is not a prominent feature of this process, even in the second case where white matter is almost universally affected. The process, except in the first case (and there might be an explanation for this fact), is characterized by a moderately severe, wide-spread microglial reaction, unaccompanied by phagocytic response and accumulation of products of degeneration. Inflammatory changes are present in the second case to a very small degree but conspicuously absent in the other two. There is nothing in the three cases to suggest vascular congestion, no evidence for vascular thrombosis. On the contrary, vascular proliferation and endothelial degeneration are striking findings in the first case.

The neuropathologic changes have almost all the features of cerebral edema as delineated by Scheinker (16)—features that he feels differentiate cerebral edema from what he calls cerebral tumefaction. These are alveolar or sieve-like appearance of nervous tissue, maximal distention of perivascular or pericellular spaces, evidence of degeneration of capillary endothelium, no increase in neuroglial fiber and the presence of only a few microglial phagocytes near the walls of some blood vessels. The major difference in our cases was the lack of vascular congestion and stasis and the fact that simple cerebral edema usually involves the white matter more than the grey.

These two facts might be interpreted as signifying that in our cases we are dealing with a different process, or something more than simple edema. Certainly, the "organization" and extent of involvement, lack of frank necrosis, especially in the first case, suggests a more chronic process, perhaps even a more complex one than that which usually results in simple cerebral edema. Since we are dealing here with what may be a new pathologic entity, it behooves us to consider the possibility of the changes observed being artefactual. We have already recognized the fact that the scattered areas of early necrosis found in the cortex and certain other areas (such as the substantia nigra and the olives and dentate nuclei) in all three cases probably represent acute anoxic changes resulting from terminal illness and agonal conditions.

It is well known that fixation and embedding may result in artefacts that superficially resemble edema. In our preparations, however, the same spongy appearance was found in sections prepared by freezing as by embedding in paraffin or celloidin. On the other hand, the regularity and extent of the vacuoles, the selectivity of the spongy degeneration for certain areas, the capillary proliferation and endothelial degeneration, and, most important, the extensive microglial reac-

tion in the second and third cases all argue against the observed changes being artefactual.

The glial response found in Cases 2 and 3, and especially the absence of it in the first case, which showed the most advanced and best developed spongy degeneration, is worthy of comment.

The age at death of these patients must be taken into consideration. The first patient was only 7 months old at death while the other two were 20 and 25 months old, respectively. Clinical evidence seems to indicate the presence of a more rapid, more extensive process in the first case, yet the glial response is absent. The reason for that may be that this child had died of intercurrent pneumonia at an earlier age. Another explanation may be the fact that very young children are "incapable" of producing a glial response. In the last 2 children, who apparently died of the primary disease, the glial response appears fresh. The glial fibers are rare and certainly nowhere in these two brains can one find the dense glial network one might expect from an old quiescent process. The glial response here is one that appears to be active and in some ways resembles that found in some of the subacute encephalitides, especially van Bogaert's subacute sclerosing leucoencephalitis.

The lack of phagocytic response and the almost total absence of "Abbau" products is probably due to the fact that demyelinization is relatively mild. This explanation, however, cannot suffice in view of the widespread distribution of actual loss of tissue in some areas. Again, here we must postulate some factor inhibiting the usual phagocytic reaction and in addition suggest the possibility of metabolic transformation of the abbau products into some material that cannot be demonstrated by the histological techniques employed. It has been suggested that perhaps such a material was present in the vacuoles and was lost during fixation. At the present time, no satisfactory explanation is available for all observed phenomena.

One condition in which the pathologic picture is similar to the one encountered in our 3 cases is one of the leucodystrophies first described by van Bogaert & Bertrand (18) under the name of "familial idiocy with spongy degeneration of the neuraxis". These cases show status spongiosus of the grey and white matter of the cerebral hemispheres as a prominent and characteristic feature, but have as another equally important characteristic an extensive, generalized dysmyelinization (10). In this disease, a profusion of giant, naked glial nuclei of the Alzheimer Type II cell are present. This disease, as the name indicates, is at least familial, probably hereditary. In our three cases the resemblance ends with the spongy degeneration. The other features are completely lacking.

Our first case was a first child with one later born normal sibling; our second patient was a fourth child with completely normal siblings, while the third patient had one older and now two younger, normal siblings. Thus far there is nothing to suggest that we are dealing with a familial or hereditary condition although the possibility cannot be ruled out at the present time.

Another group of diseases to consider especially in the first and third cases, are the poliodystrophies reported by Alpers, (1), by Ford *et al.* (5), by Christensen & Krabbe (2), and others. Superficially our Cases 1 and 3 would resemble this condi-

tion except for the lack of familial incidence. It is of interest that DeJong & Bebin (3) reported a case showing a combination of true poliodystrophy with leucodystrophy of the status spongiosus type.

The leucodystrophies and the poliodystrophies are almost certainly diseases characterized by abnormal metabolism, especially the former which are closely related to the lipidoses. In phenylketonuria, another dysmetabolic condition which severely affects the central nervous system, even though the type of demyelinization is similar to the one found here (15), there are as a rule no changes comparable to the spongy degeneration found in our case with the one major exception of the case reported by Sander (15). Lichtenstein (7) has pointed out that in the status spongiosus of pernicious anemia, the astroglial response is often inadequate and very often the glial cells perish. The deficiency of the glial response is believed to be a factor in the formation of the sieve-like or honey-comb areas so commonly termed status spongiosus. Einarson & Neel (4) have postulated the necessity of an enzymatic factor to be present in the tissue in order to summon a glial response in the presence of injury. Rather than suggest a factor in a mechanistic sense, Poser & van Bogaert (13) preferred to look upon this as a stage in the organism's response to injury without invoking the presence of a specific "substance" or factor. Be it as it may, primary injury to the glial system rather than to the ground substance may well be an important factor in the pathogenesis of the disease in our three cases. It is interesting to speculate that here, as in other instances of status spongiosus, a metabolic disturbance might be the basic pathogenetic defect.

From the pathological findings, it is impossible, at the present time, to offer an explanation for the clinical picture. Superficially the clinical illness resembles subacute sclerosing leucoencephalitis of van Bogaert which, interestingly enough, also has a characteristic electroencephalographic picture. Yet, there can be no question that it is the anatomical involvement, not the type of tissue damage, that determines the clinical signs and symptoms. Undoubtedly the same can be said in our three cases. The fact that in the first case it is the grey matter that is primarily involved, that in the second it is the white matter which bears the brunt of injury, while in the third both grey and white matter are involved is puzzling in view of the similarity of the clinical picture, and also in terms of the etiology of the disease. However, in all three cases the disease is widespread and essentially the same anatomical structures are involved, either directly by cell loss, or indirectly by interruption of the nerve fibers issuing from spared ganglion cells.

Work has been in progress during the past few years trying to demonstrate in children with infantile spasms and hypsarhythmia abnormal urinary metabolites which may be related to an underlying biochemical defect. This line of research is promising but not yet conclusive.

Summary

Detailed brain autopsy reports on three children with infantile spasms and progressive physical and mental retardation are reported. The findings in these cases

are different from those described in other well-defined disease entities but they are similar to each other. The outstanding features are chronic edema and spongy degeneration with but little glial reaction and little evidence of repair. In the first case, the grey matter was primarily involved; in the second case, the changes were mainly of the white matter; and the third case was mixed in distribution. This syndrome is not one of primary demyelinization. It is possible that an enzymatic defect is responsible for the poor glial and phagocytic response in these brains; this would be compatible with the clinical impression that many, if not all, children with this clinical syndrome suffer from a disease of metabolic origin.

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Blood Level of Cerebrosides in Gaucher's Disease[1]

by PER OLOF HILLBORG and LARS SVENNERHOLM

Gaucher's disease is a heredofamilial disorder characterized by a disturbance in the cellular metabolism of cerebrosides. These lipids are accumulated in large, pale cells (Gaucher cells) which arise from the reticuloendothelial cells in all parts of the body. Many theories have been advanced concerning the cause of the disturbed cerebroside metabolism in Gaucher's disease. Pick (6) formulated the theory of a primary disturbance of the intermediary lipid metabolism which expresses itself in an accumulation of cerebrosides in the blood plasma and secondarily to a storage of the abnormal lipids in the reticuloendothelial cells of the spleen, liver and bone marrow. This view has been strongly criticized by Thannhauser and coworkers (9). They assume a dysfunction of the reticulum cells themselves as a result of an enzymatic disturbance within the cells. This leads to an increased biosynthesis of cerebrosides and simultaneously to a storage of these substances within the cells. Thannhauser and coworkers have mainly based their theory on negative tests for cerebrosides in normal serum as well as in serum of patients with Gaucher's disease.

Svennerholm & Svennerholm have recently shown (7) that earlier methods for the estimation of cerebrosides in blood are unreliable. Using a new method they have determined the cerebroside level of normal sera. We have used the same method and have analysed sera obtained from six splenectomised patients with Gaucher's disease. In all the content of cerebrosides was increased.

Material

The six patients live in Norrbotten County of northern Sweden and have been treated at the Department of Pediatrics, County Hospital of Boden. All blood samples for cerebroside analysis were obtained in 1958.

Some of their clinical and laboratory data are summarized in Table 1. In all the patients the first symptom was enlargement of the spleen at or before the age of one year. All six developed anemia, four of them leucopenia and five thrombocytopenia. Splenectomy was performed from one to eleven years after the onset of the disease. In all cases the spleen contained typical Gaucher cells. Only one spleen was sent for chemical analysis. The cerebroside content was 4% of the dry weight.

Anemia was not much influenced following the operation, but in all cases a pronounced leucocytosis developed which persisted at least five years after operation. The thrombocyte count returned to normal after operation in all cases.

X-ray examinations of the skeleton were

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TABLE 1.

Case	Splenomegaly detected at age	Splenectomy at age	Blood values: Preoperative lowest value	Blood values: Mean value two first years after operation	Blood values: At the time for cerebroside analysis	Bone change: Preoperative	Bone change: Postoperative after years
1. A. Å. born 1946	½ year	1½ year	Hb.: 57 % Wbc: 3.2 × 1000 Platelets: 102 × 1000	67 (64–76) 16.8 (8.4–31.7) 301 (130–580) $n = 10$	73 10 412 11 yrs postop.	—	Hip joint 1½ Femur metaphysis 2½
2. J. J. born 1947	½	7	Hb: 46 % Wbc: 3 × 1000 Platelets 59 × 1000	70 (62–80) 18.2 (5.0–29.6) 283 (187–444) $n = 9$	70 21.8 250 4 yrs postop.	—	Hip joint 2 Femur metaphysis 2
3. L. J. born 1951 sister of Case 2	1	3	Hb: 63 % Wbc: 2.4 × 1000 Platelets: 72 × 1000	70 (65–80) 24.9 (11.1–37.2) 364 (207–527) $n = 4$	71 21.2 292 4 yrs postop.	—	Femur metaphysis 2
4. S. Ö. born 1952	½	1½	Hb: 49 % Wbc: 4.3 × 1000 Platelets: 69 × 1000	67 (61–75) 21.1 (11.2–31.5) 330 (140–580) $n = 13$	65 14.7 348 4½ yrs portop.	—	Hip joint 3½ Femur metaphysis 3½
5. J. Ö. born 1954 brother of Case 4	½	1½	Hb: 53 % Wbc: 2.4 × 1000 Platelets: 94 × 1000	65 (54–79) 13.2 (10.5–16.8) 339 (148–566) $n = 9$	61 12 367 2½ yrs postop.	—	Femur metaphysis 2½
6. K. J. born 1940	1	12	Hb: 66 % Wbc: 7.4 × 1000 Platelets: 152 × 1000	88 (74–101) 21.0 (15.7–26.2) 232 (183–282) $n = 4$	73 9.9 319 6½ yrs postop.	—	Spin.column 1 Femur metaphysis 4

normal preoperatively. Shortly after splenectomy changes were observed. A detailed report of these changes will be reported (Hillborg, unpublished).

Method

The method described by Svennerholm & Svennerholm (7) for the determination of cerebrosides in plasma was followed in detail: venous blood was drawn into centrifuge tubes containing sodium citrate and centrifuged. The *plasma* was pipetted off after centrifugation and treated as described (7). The *blood cells* were resuspended in two volumes of physiological saline and centrifuged. The supernatant was decanted off and the washing was repeated twice. After the last decantation the remaining cells were packed by centrifugation at 3.000 rpm for 3 minutes. A pipette with a wide tip was brought to the bottom of the centrifuge tube and 5 ml packed cells was transferred to a small flask. Five ml physiological saline was added and the flask was thoroughly swirled. Two ml cell suspension was dropped into 50 ml boiling methanol. The solution was filtered and the precipitate was washed twice with 5 ml portions of warm methanol. The combined filtrates were evaporated *in vacuo* and redissolved in 20 ml chloroform-methanol 2:1, v/v. 5 ml 0.1 % saline was added and the content mixed by shaking. Then the procedure applied for plasma extracts was followed.—All analyses were performed at least in duplicate.

Results

The results of the analyses appear in Table 2. Also shown is the mean value for plasma and red blood cell cerebrosides in 25 normal subjects aged 28–82 years. It is evident that the plasma cerebroside level in the patients with Gaucher's disease is nearly twice as high as the normal mean. Although the normal subjects were older than our cases, analysis of plasma from normal children indicate that their cerebroside level is somewhat lower than in adults. The possibility that the raised level of cerebrosides was a result of the splenectomy as such was excluded by analyzing plasma of two patients splenectomised after traumatic rupture of the spleen. Their cerebroside levels were normal, 3.9 and 4.4 mg/100 ml, respectively.

It is difficult to draw any conclusions from the cerebroside analyses of the red cells. The normal material comprises only 10 cases and the variations are large in our five cases analysed. In three of the cases the cerebroside content was normal but it was raised in two of them.

TABLE 2. *Cerebroside content of plasma and red blood cells*

Case	Cerebrocides mg/100 ml	
	Plasma	Red blood cells
1. A. Å.	9.9	39
2. J. J.	9.7	25
3. L. J.	8.1	—
4. S. Ö.	7.4	26
5. J. Ö.	5.9	24
6. K. J.	7.5	53
Normals age 28–82 years	4.36 ± 0.18 ($n = 25$)	22–30 ($n = 10$)

Discussion

As shown by Svennerholm (8) and Brückner (1) the cerebrosides are mainly membrane lipids and occur in all parenchymatous organs in about the same amount as in the fetal brain. Under normal conditions galactose is the only sugar which can be identified in the cerebrosides, but in Gaucher's disease the cerebrosides accumulated in the reticuloendothelial organs mainly contain glucose. It is probable that in Gaucher's disease glucocerebrosides are formed in all organs. The abnormal cerebrosides cannot undergo metabolic breakdown by the usual pathways. As they are not metabolised in the cells where they are formed, they are released to the intercellular space and blood and then taken up and stored in the reticulum cells.

The spleen is the main storage place for the gluco-cerebrosides. If the hypothesis above is valid the accumulation of glucocerebrosides in other organs containing reticulo-endothelial cells must be accelerated after splenectomy, primarily in the liver and bone marrow. Many authors are of the opinion that splenectomy does not hasten bone involvement in Gaucher's disease (Logan (2)). However, Müller (3), Myers (4) and Pack *et al.* (5) and Ullrich have observed cases in which a rapid progress of skeletal changes occurred soon after splenectomy.

Several authors state that the prognosis in Gaucher's disease depends on the age at which the first symptoms arise. In this respect the present case material is unusually homogeneous; all patients developed the first symptom of the disease at or before the age of one year. One might expect evidence of skeletal involvement to appear at about the same age, whereas in fact

bone changes occurred at quite different ages. But in all patients they appeared within four years after splenectomy. This observation makes it likely that splenectomy leads to an accelerated deposition of cerebrosides in the bone marrow.

Summary

The cerebroside content of blood in six patients with chronic Gaucher's disease, all splenectomized, has been analysed.

The plasma level was markedly increased in all. The present investigation supports the hypothesis of Pick that there is a generalised disorder of the cerebroside metabolism in Gaucher's disease which expresses itself in an accumulation of cerebrosides in the plasma and, secondarily, leads to storage of the abnormal lipids in the reticulum cells.

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A Study of Group A Hemolytic Streptococcus Carriers Among School Children

Part I. Material, Methods and Results of the Studies

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The possibility of preventing rheumatic fever by early treatment of streptococcal pharyngitis has led to epidemiological studies on the spread of the group A hemolytic streptococcus.

Most of these investigations have been carried out on members of the armed forces. Thus they were performed upon a large number of methodically followed up and closely studied subjects, and yielded, under the circumstances, exceptionally valuable information. Their results, however, are only valid for a well defined population group, viz. young adults living in closed communities under conditions particularly favourable for the spread of infections. Moreover, most of these investigations were made during the course of epidemics.

For these reasons it is particularly interesting to study the epidemiology of streptococcal infections in communities of children, especially in schools. The conditions of study here are very different from those met with in the armed forces not only on account of the age of the subjects but also because the group life of school children is of a less permanent kind and the number of new infections brought in from outside is not negligible.

Three types of investigations have been made among children. Some were undertaken during *an epidemic*, their principal aim being (as in the case of the one by Russel (22) and that of Poskanzer) (16) to study the most effective measures of stopping the epidemic. Others were undertaken with a view to detecting *streptococcal throat infections*; that is among all the sore throats and pharyngitis oc-

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curring in school-children, to identify those due to the group A hemolytic streptococcus, and therefore likely to benefit by treatment with antibiotics. The original study of Bunn & Bennet (2) along these lines was followed by a series of similar ones. Finally, a third type of investigation was that of a *systematic search for bacterial carriers* unconnected with any epidemic or even any infection in normal, class attending, and apparently healthy school children. Several surveys of this kind were made in the United States (4, 15, 17, 23) and Great Britain (10). The present study belongs to this third category: it consisted in choosing a group of 241 school children of the same age group, and taking throat swabs systematically from these 241 children every fortnight for two consecutive school years, at the same time collecting all information likely to explain the occurrence of a streptococcal infection in any one of them. Such an investigation permits only the detection of germ carriers (temporary or chronic), and possibly of subjects with clinically apparent infection; it cannot distinguish a healthy carrier from a child with subclinical disease, revealed solely by immunological tests.

Material and Methods

I—*Selection of subjects*

The town where our survey was made, was chosen because of the facilities granted by the school and municipal authorities. It is a suburban township of some 40,000 inhabitants, which comprises several school units. The unit chosen for the survey was selected for practical reasons (easy access, interior arrangements suitable for swabbing) and also because its population seemed to present socio-economic conditions very favourable for the purposes of the investigation.

The children followed during the whole period of the survey were attending the first year medium course to begin with. Three forms of boys and three forms of girls belonging to the same school unit, but occupying, within the latter, separate and quite different premises, were selected. The boys occupied a building of recent construction, well-lit and aired, relatively spacious and with tiled floors. The girls, housed in an old, much darker building, with wooden floors, were much more crowded together and definitely less favoured from the health point of view.

The six forms selected were examined according to the method described below from February (beginning of the enquiry) to the end of June 1957 (beginning of Summer School Holidays). During the second year of the survey (from November 1957 to the end of June 1958), the children of the corresponding six forms of the 2nd year Medium Course, which comprised most of the children examined during the previous year, were followed. The difference, inevitable in any survey of this kind, is due to the reorganization of the forms and the movements of population. Subjects studied were as follows:

—during the first year, 217 children of whom there were 106 boys, and 111 girls,
—during the second year, 228 children of whom there were 104 boys, and 124 girls.

Ninety of the boys and 105 of the girls followed during the second year had been followed during the first year.

The average age of the children was 10 years, ranging from 8 years to 13 years 4 months.

Before the survey was begun, the total contingent of children was divided into two comparable groups by drawing lots, using a table of random figures. These 2 groups were established in order to study the influence of various techniques.

The cooperation of the teachers, the families of the children and the physicians of the town was secured by numerous personal contacts,

talks and lectures explaining the aims pursued and the methods to be used. This preliminary preparation, in which we were actively assisted by the municipal and academic authorities and by the School Health Services, enabled us to carry out our investigation without any difficulty and in an atmosphere of understanding which greatly facilitated our task.

II—*Swabbings*

In so far as was possible, throat swabs were taken from all the children every fortnight, except during the long summer holidays. In some cases the swabs had to be taken after an interval of 3 weeks.

For practical reasons the swabs were taken in the morning from 10 to 11 a.m., three forms a day for 2 days running. To avoid a possible cause of error, the different forms were attended to in the same order each time, fixed at random at the outset of the survey. The class teacher was swabbed at the same time as his or her class.

Except in a few cases, the swabs were taken by the same person throughout the enquiry. In addition, the child was asked a few questions about any recent illness in himself or in the persons living at his home, or any current treatment (antibiotics) likely to affect the findings of the swab. Each of the children underwent a brief clinical examination of the throat and the lymph-glands of the truncus jugularis.

All this information was recorded on a filing card on which the bacteriological results were subsequently added.

Twenty-five sets of throat swabs were thus taken from February 1957 to June 1958.

Beside these routine swabbings, a welfare worker or a school nurse took, on every working day, a throat swab of any child of the followed up group who had been absent from morning class. These swabs, sent to the laboratory the same morning and examined directly without enrichment media, were meant to establish any acute streptococcal infection that might have occurred among the children.

III—*Medico-social study*

For each regularly followed child a file was constituted comprising: the 25 cards drawn up during the successive swabbings mentioned above. A set of answers to 98 codified questions forming a relatively searching medico-social enquiry. These questions bear upon the following matters: civil status, physical information of genetic nature (colour of the eyes and hair, race, filiation, etc. ...), personal record of the subject (with special reference to streptococcal, rheumatic and allergic previous history), past record of all persons living in the child's home, social level appraised in terms of the father's occupation (and possibly of the other persons working in the household) and in terms of the main expenditure headings, housing conditions (especially the degree of hygiene and overcrowding), special factors of contamination due to the occupations of the child or of his parents.

This record was completed during the first four months of the study, partly by the school physician, partly by the welfare worker.

These results were coded and transcribed on perforated cards so as to allow as full a mechanographic exploitation as possible later on.

IV—*The technique of swabbing*

Considering the way in which the swabs were taken to be of paramount importance, we made it a point to strictly observe the following rules: (1) Since swabbings cannot be taken fasting in our school children, the throat swab was taken at least 2 hours after breakfast. (2) The swab was taken after the child had been made to swallow if his throat was full of saliva. (3) The use of a sterilized metal tongue spatula was the rule, not only in order to expose the site of the swabbing properly but also to prevent contamination by the germs of the tongue and the saliva. (4) The swabbing was performed by the same operator throughout the study. (5) The swabbing was performed by firmly rubbing

the right tonsil, the right anterior arch, the contour of the uvula, the left anterior arch, the left tonsil, in that order, ending the procedure by touching the posterior wall of the cavum if it was not covered with muco-pus of nasal origin. (6) The content of the applicator was either immediately put on a blood agar plate or was introduced into a preservation and enrichment medium prior to being taken to the laboratory.

V—*Isolation*

A. The isolation itself was always done in the same way, but three different preliminary procedures were used:

(a) *direct isolation*: the blood agar plates were brought to the place of swabbing. The throat swab was put on blood agar on the spot. The plates thus seeded were taken in a metal carrier back to the laboratory where, about one hour later, they were placed in an incubator at 37°C.

(b) *isolation after passage of the throat swab in an agar selective medium* (containing crystal violet and sodium azide in the proportions suggested by Holmes (9). This medium was contained in tubes of 15 cm by 1 cm, 2 ml being poured into each tube. As soon as the swab had been taken, it was introduced into the agar medium in such a way that its wad of cotton was buried but the part of the metal stem which had been in contact with the operator's fingers emerged above the cotton stoppering the tube. Taken to the laboratory without any special precautions, the tubes were left at room temperature for 24 hours. At that time our main concern was to try out a transport medium which, whilst sparing the streptococci in the swab, would inhibit to the maximum the growth of other organisms likely to develop at the expense of the streptococcus.

(c) *isolation after passage of the throat swab in a selective medium* of the same composition as the previous one but without agar and therefore *fluid*. One advantage of this medium is to allow dispersal of the organisms by shaking; it further yields a more abundant culture. It presents, however, the disadvantage of making transport more difficult. Assuming that, in selective media, inhibiting substances act especially by prolonging the latent phase of the organisms to be evicted during the period the organism sought for is beginning to develop, we limited the incubation at 37°C to six hours. The isolation was performed at the end of these 6 hours.

B. Isolation proper: Isolation was always done on Petri dishes of a diameter of 10 cm containing horse blood agar. The throat swab was pressed on to a small surface of the dish and the inoculum was then spread out in quadrants.

After 20 hours of incubation at 37°C a first reading of the plates was done. Colonies likely to be hemolytic streptococci, judging from the presence of a zone of complete clear hemolysis or imperfect but non green turning hemolysis[1], as well as from their size and appearance, could be taken up at once. Doubtful or negative plates were put back in the incubator and were submitted to a second reading 24 hours later, i.e. after incubating for 48 hours, at the end of which time certain colonies might become apparent or only then seem to be suspect.

In case a plate had been contaminated by a Proteus (or any other contaminating mobile organism), a second isolation was made by reseeding the original inoculum on an incubator-dried blood-agar plate, which was again dried after the seeding and placed in the incubator with about ten drops of absolute alcohol in its cover.

VI—*Purification*

Because of the many examinations required and the limited possibilites of our laboratory, only a single colony was further purified; this was done by another isolation,

[1] The frequently used term "beta hemolysis" should be reserved for hemolysis obtained by mass culture. In surface seedings the term "clear hemolysis" seems preferable for designating non green-turning hemolysis.

the colony being spread out on blood agar in the way already described.

As, however, the original isolation plate was not thrown away until the organism had been identified, one or several other colonies could be taken up from it, if, on comparing the 2 plates (isolation and purification), the purified colonies seemed different from the suspect colony.

VII—*Identification*

A colony thus purified was collected by capillary aspiration with a slightly bent micropipette and seeded in a hemolysis tube containing 2 ml of blood broth. After incubation for 6 to 8 hours, the growth of the culture was noted as well as any lysis of the blood cells, which would indicate the presence of a soluble hemolysine.

The purity of this culture was checked on one quarter of a blood-agar plate (the 3 other quarters serving for 3 other cultures).

If the culture proved to be pure, 1 or 2 drops were taken to seed 20 to 30 ml of glucose broth, and placed in an incubator for 24 hours; the germs collected by centrifuging of this broth were used to make an extract for grouping according to the technique of Lancefield.

More often than not, when the suspect colony seemed uncontaminated on the first isolation plate, we seeded it directly in blood broth without passing through the purification stage, subsequently resuming purification if necessary. This enabled us to identify the organisms 24 hours earlier.

VIII—*Preservation*

A. *Solid media for Petri dishes.* Since the bottom of commercially available Petri dishes is seldom flat, blood agar poured on to them is not of the same depth throughout; this renders the interpretation of the hemolytic zone and of its colour (alpha, beta or gamma) especially difficult. To overcome this difficulty it seemed best to make the bottom of the plate even by first pouring on to it a thin layer of ordinary colourless agar; subsequently, it is thus possible to obtain a layer of blood-agar of equal depth over the whole surface of this dish. We therefore used 2 media for our Petri dishes:

(1) *basic agar* the composition of which is as follows: for one litre of distilled water: Sodium Chloride 9 g, Agar-agar 200 g.

(2) *blood agar* prepared by adding 4 % of defibrinated horse blood[1] to a buffered broth, without glucose. It seems particularly important to use broth without glucose, as many trials made in the laboratory have shown that glucose can modify the hemolytic power of the streptococci. This buffered medium is prepared as follows: for one litre of distilled water: Liebig extract 5 g, Fibrin peptone 20 g, NaCl 2.5 g. Adjust the pH to 7.8 and add: Monopotassium phosphate 0.7 g, Disodium phosphate (12 H_2O) 8.3 g. Filter on hard Durieux, precipitate for 20 minutes at 120°C, leave for 4 hours, pour off and add: Agar-agar 15 g. Divide out and sterilize for 20 minutes at 115°C.

B. *Selective enrichment media.* Three different media were used successively:

(1) *A fluid medium* adapted from that of Pike (*Technique 2*). Its basic element is a meat extract broth described in the preceding paragraph to which are added, for one litre of broth: Sodium azide 2 g, Crystal violet (1/400 solution) 1 ml, Hemolysed horse red blood cells 30 ml.

(2) *Agar medium (Technique 3).* This selective medium is adapted from the one described by Holmes (9). For its preparation is also used as a basis the buffered meat extract broth (see preceding paragraph). After decantation add to it, for one litre of buffered broth: Glucose 4 g, Aqueous solution of crystal violet (1/1000) 1 ml, Aqueous solution of Sodium azide (1/16) 1 ml, Agar-agar 15 g. After dividing out into 30 ml vials and sterilising, add, taking sterile precautions,

[1] It is a well-known fact that compared to sheep blood, horse blood has the disadvantage of being hemolysed by *haemophilus haemolyticus*. We have nevertheless used horse blood for practical reasons, taking care to identify microscopically all hemolytic organisms which would not grow in ordinary broth. We have, of course, never used human blood (which may contain an antistreptolysin).

to each vial 1.5 ml of defibrinated horse blood.

(3) *Fluid selective medium (Technique 4).* This medium is the same as the previous one but without agar-agar; having noticed that cultures in this medium developed slowly, some yeast autolysate was added, a precaution which corrected the above-mentioned drawback. The formula used is as follows: Distilled water 1000 ml, Meat extract (Liebig) 5 g, Fibrin peptone 20 g, NaCl 2.5 g, Barm autolysate 3 g. Adjust the pH 7.8 and add: Monopotassium phosphate 0.7 g, Disodium phosphate (12 H_2O) 8.3 g. Filter on hard Durieux, precipitate for 20 minutes at 120°C, allow to settle for 4 hours, pour off and add: Glucose 4 g, Aqueous solution of crystal violet (1/1000) 1 ml, Aqueous solution of Sodium azide (1/12) 1 ml. Divide out into vials of 30 ml; sterilize for 20 minutes at 115°C. Before using add 1.5 ml of defibrinated horse blood.

C. *Culture media.* The colonies isolated from blood-agar plates were cultured in a buffered broth without glucose but containing 3 % defibrinated horse blood. This medium seemed to present the following advantages: (1) more rapid development of colonies, (2) demonstration of the production by the organism of a diffusible hemolytic toxin, a characteristic essential for the recognition of group A, C and G streptococci, all three of which produce soluble streptolysins.

For the preparation of the Lancefield (12) extract the culture was made in a buffered broth containing glucose and without added blood.

Results

Our first objective was to compare the merits of different techniques for the isolation of group A hemolytic streptococci.

1—*Comparison of techniques of throat swabbing*

We took two swabs from each of 185 children on the same day. The first swabbings which we called "Properly taken throat swabs" were carried out with the previously indicated precautions; the second designated as "Inadequately taken throat swabs" were performed without observing these precautions.

TABLE 1.

Method	Number of throatswabs	Number of A	% of A
"Properly taken throat-swabs"	185	38	20.5
"Inadequately taken throat-swabs"	185	15	7.5

The results obtained in these two series are indicated in Table 1.

It is thus evident that by taking throat swabs according to a proper technique, other things being equal, there are three times as many chances of discovering the hemolytic streptococcus in the same group of people.

(We tried out this experiment during the course of a tentative study in a community of mentally retarded children in whom it was difficult to perform the swabbing correctly. These results led us to abandon this undertaking.)

II—*Comparison of isolation techniques*

The first selective medium which we studied was adapted from that of Pike (13). We tried it out on three sets of throat swabs taken in February and March 1957, comparing our results with those which we had obtained with the technique of direct isolation on a blood-agar plate (Technique 1). The results obtained with these two techniques are indicated in Table 2 and Fig. 1.

The persistence of negative results with

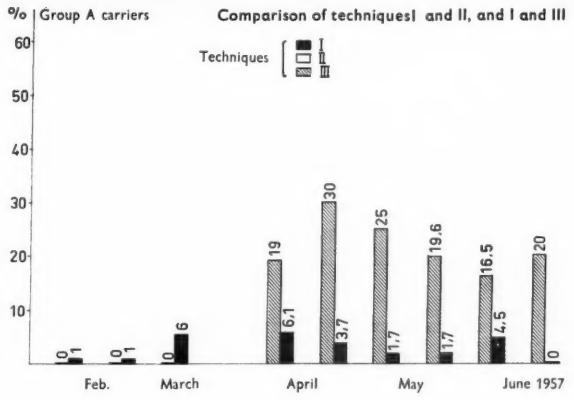

Fig. 1.

TABLE 2.

Date	Techniques	No. of swabbings	No. of A	% of A
2–57	Technique 1	112	1	1
	Technique 2	98	0	0
2–57	Technique 1	112	1	1
	Technique 2	98	0	0
3–57	Technique 1	103	6	6
	Technique 2	95	0	0

Technique 2 soon induced us to abandon it.

Between April and June 1957 we made a comparative study of two isolation techniques: (1) the technique of direct isolation on blood-agar without passage through a selective medium (Technique 1). (2) the technique of isolation after passage of the swab in an agar medium adapted from that described by Holmes (9) (Technique 3).

The results are indicated in Table 3 and Fig. 1.

In comparing the percentages of group A streptococcus carriers obtained with the two techniques, it was noted that there were 10 times as many carriers detected when the agar selective medium derived from Holmes' was used than by the method of direct isolation on blood-agar (Fig. 1).

It was therefore this technique of isolation after passage of the throat-swab in agar selective medium that we used in all our children from November 1957 to May 1958. During the months of May and June

TABLE 3.

Date	Techniques	No. of swabbings	No. of A	% of A
9/4/57	Technique 1	113	7	6.1
	Technique 3	101	19	19
29/4/57	Technique 1	108	4	3.7
	Technique 3	97	30	30
14/5/57	Technique 1	116	2	1.7
	Technique 3	96	24	25
28/5/57	Technique 1	113	2	1.7
	Technique 3	97	19	19.6
11/6/57	Technique 1	110	5	4.5
	Technique 3	97	16	16.5
25/6/57	Technique 1	103	0	0
	Technique 3	82	17	20

1958 we tried to improve further on this technique, and in Groups 1 and 2 of children, we compared the results obtained with the following two isolation techniques: (1) isolation after passage of the throat-swab in the "agar" medium derived from that described by Holmes (Technique 3). (2) isolation after passage of the throat-swab in the same "non agar" medium with added barm autolysate (Technique 4).

Table 4 sums up the results obtained with these two techniques:

TABLE 4.

Date	Techniques	No. of swabbings	No. of A	% of A
12/5/58	Technique 3	58	7	12
	Technique 4	63	15	24
13/5/58	Technique 3	37	3	8
	Technique 4	59	8	13
3/6/58	Technique 3	57	7	12
	Technique 4	51	7	13.5
4/6/58	Technique 3	39	4	10.3
	Technique 4	64	8	12.5

The results are represented in Fig. 2.

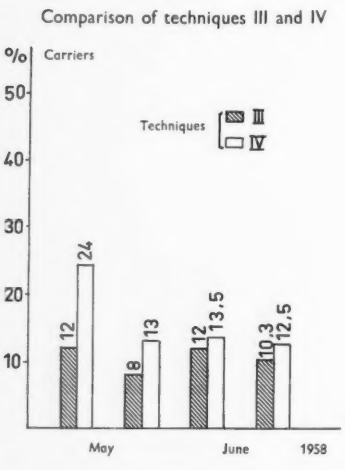

Fig. 2.

The differences between the results of these two techniques are certainly less striking than those which we had seen between Techniques 1 and 3; nevertheless, statistical study shows that this difference is significant, and that Technique 4 may be regarded as superior to Technique 3 for the detection of group A streptococci in throat swabs.

III—*Variation of the carrier-rate during the course of the year*

From the beginning to the end of this study the throatswabs taken from the children of Group 2 were examined by Technique 2, and later by Technique 3; the throat swabs taken from the children of Group 1 were examined by means of 3

TABLE 5.

Date	Group of children	Total number of swabbings	Total number of A carriers	% of A
April 57	2	101	19	19
	2	97	30	30
May 57	2	96	24	25
	2	97	19	19.6
June 57	2	97	16	16.5
	2	82	17	20
Nov. 57	2 + 1	223	38	17.5
	2 + 1	223	36	16.1
Dec. 57	2 + 1	226	37	16.3
	2 + 1	211	34	16.1
Jan. 58	2 + 1	224	22	19.8
	2 + 1	222	22	9.9
Febr. 58	2 + 1	224	15	6.7
	2 + 1	223	38	17
March 58	2 + 1	230	43	18.7
	2 + 1	221	31	18.5
April 58	2 + 1	218	57	26.1
	2 + 1	220	43	19.6
May 58	2	95	10	10.5
June 58	2	96	11	11.4

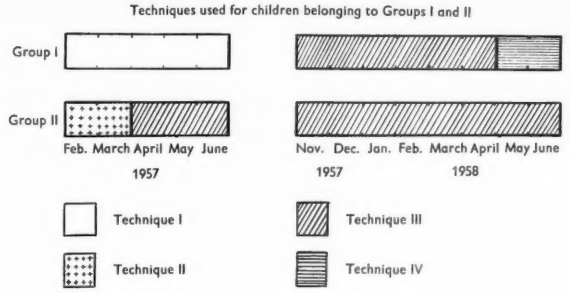

Fig. 3.

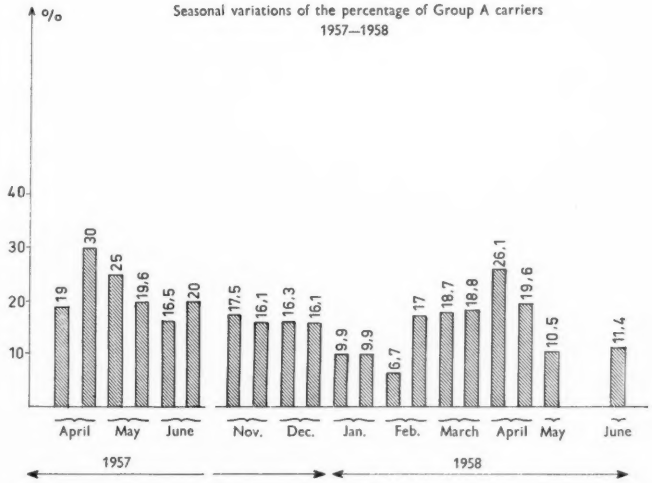

Fig. 4.

different techniques at different times: between February and July 1957: Technique 1. From November 1957 to May 1958: Technique 3. During May and June 1958: Technique 4 (Fig. 3).

To study the variations in the percentage of carriers in the course of the year, we should therefore take into consideration only the swabbings which had been treated with the same isolation methods. This led us to eliminate from the study the swabbings studied with Techniques 2 and 4. Our analysis thus covers all the children of Group II plus those of Group I, the latter only for the period from November 1957 to May 1958.

The results obtained are given in Table 5 and Fig. 4.

In studying this school community with the same technique we have thus observed variations in the percentages of group A streptococci carriers ranging from

6.7 % to 30 %. During this period, from April 1957 to the end of December 1957, the maximum number of carriers was found towards the end of April with a rate of about 30 %. In the first 6 months of 1958 it was again in the month of April that we found the largest proportion of carriers, i.e. 26.1 %.

IV—*Curve of acquisitions and cumulative percentage*

In each successive series of throat swabs, we found that group A hemolytic streptococci appeared in children in whom we had been hitherto unable to demonstrate their presence. To study the course in time of this phenomenon, which can be called a "curve of acquisitions", we had to select only those of our children who had not been absent more than 5 times from the 24 throat swabbing sessions effected. Statistical study has shown that with a higher number of absences the results were liable to be erroneous. We have thus studied the results obtained in 89 children only. The cumulative percentage represents

TABLE 6.

No. of swabbing	Date	Number of aquisition	Total no. of carriers at least once	Cumulative %
IV	9/4/57	13	13	14.6
VI	29/4/57	15	28	31.4
VII	14/5/57	2	30	33.7
VIII	28/5/57	3	33	37
IX	11/6/57	2	35	39.3
X	25/6/57	1	36	40.4
XI	5/11/57	6	42	47.2
XII	26/11/57	2	44	49.4
XIII	3/12/57	3	47	52.8
XIV	17/12/57	0	47	52.8
XV	7/1/58	1	48	53.9
XVI	21/1/58	1	49	55
XVII	4/2/58	1	50	56.1
XVIII	25/2/58	1	51	57.3
XIX	11/3/58	2	53	59.5
XX	25/3/58	0	53	59.5
XXI	15/4/58	0	53	59.5
XXII	29/4/58	1	54	60.6
XXIII	13/5/58	1	55	61.7
XXIV	3/6/58	0	55	61.7

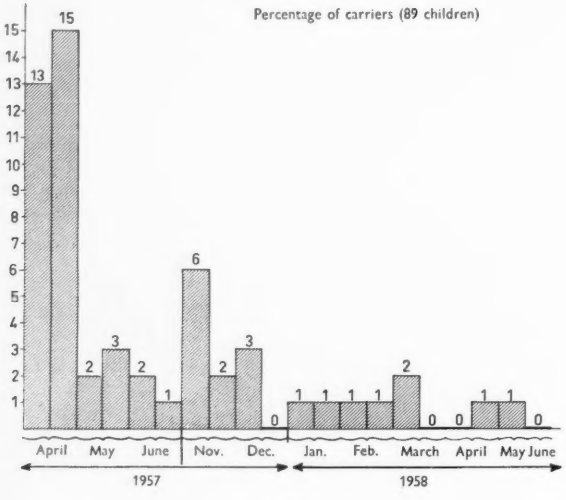

Fig. 5.

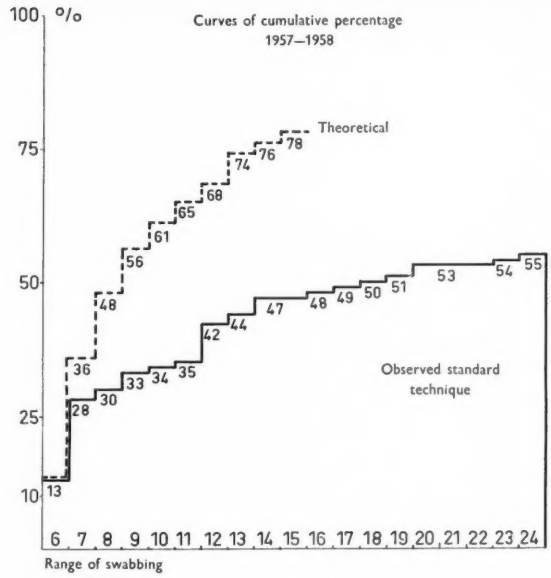

Fig. 6.

the percentage of children who were found to be carriers of group A hemolytic streptococci at least once at a given date: Table 6 represents the number of acquisitions and the cumulative percentage for each series of throat swabs.

These results are graphically represented in Fig. 5 and 6.

Thus, within three months of study the cumulative percentage rises from 13.6 to 40.4 % reaching 61.7 % at the end of 15 months of study.

V—*Prolonged carriers*

When the results of successive throat swabs are transcribed on to a table where each child is noted individually, it is seen that the carriers present two kinds of behaviour: in some of them, the organism is found occasionally in a single throat swab or in a few consecutive ones. In others, on the contrary, it is found with great regularity either in the course of many consecutive swabbings or again, after a short interruption.

We were able to have the strains, isolated from some of the children of the latter category, typed (prolonged carriers).

The result of these examinations is indicated in Table 7. It is to be noted that the typing was done chiefly by agglutination, precipitation being performed only if necessary.

The study of this table leads to the following conclusions: (1) the prolonged carriers themselves display two different kinds of behaviour: some of them keep the same type of streptococcus for a very long

TABLE 7.

12 = Streptococcal type identified by agglutination technique; $\underline{12}$ = Streptococcal type identified by precipitation technique; S_{15} = Series of streptococcal types identified by agglutination; NT = Group A streptococcus not typable; + = Group A streptococcus, strain lost before typing; —— = Carriage of the same type, proved; – – – = Carriage of the same type, supposed; / = Change of type.

Carrier's code number	1957							Summer holidays	1958																
	Range of swabbings								Range of swabbings																
	IV	V	VI	VII	VIII	IX	X		XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII	XXIV	XXV
10				9 / 12					1														
21				8*					B* – – – B*														
26											11 – – – – 11 – – – – – – – – – 11 — 11												
31				9 — 9 — 9 — 9																			
38				$\underline{19}$					19/ 9 — 9 — 9									1					
50		11 — 11 — 11 – – – – – 11														8 – – – – – – 8 / imp*/8 — 8							
52									28/14 – – – – – – – 14														
55		2 – – – – – – – – – – 2																					
64			11 – – – – – – – – – 11						1 — 1 — 1 — 1 — 1 — 1 — 1 — 1 – – – – – 1 — 1 — 1 — 1														
70				11											12 — 12 – – – – – 12 — 12 — 12								
81									12–12 – – – – 12					NT		12 — 12 — 12 – – – – – – 12							
102		B* — B* — B* — B* — B*							B* — B* — B* — B*							B* — B* — B* – – – – – – – – B*							
103				1 – – + – – 28 / 1					2							/ 1							
111		9 — 9 — 9 – – – – – – 9 — 9																					
116				12 / 44 – – – – / 12											12								
120		12 – – – – / 28 / 12 — 12																					
131		25 / 9 – – – – – – 9																					
134						4			B*														
136		NT 2 – – – – – – – – – 2							2 – – – – 2 – – – – – 2 — 2 — 2 — 2 — 2 — 2 — 2 – – – – – – 2 — 2 — 2														
146									S_{15}—S_{15} – – – S_{15}—S_{15}—S_{15} – – – – – S_{15} / S_{17}/S_{15} – – – – /S_{17}														
153									B* / 9 – – – – – – – – – – – 9 — 9														
158			6 — 6 — 6						6 – – – – – – – – – – – – 6 – – – – – – 6														
159			11 / 9 – – – – – – 9 — 9						35 / S_{15}/12 — 12 — 12 — 12 – – – – – – / B* / 12 – – – – $\underline{12}$ – – – – – – 12														
166			12 / B*													$\underline{28}$ / 3							
169				44					+ – – – – 12 – – – – – – – – – – – – – – – – 12														
175			$\underline{28}$ – – – – – – $\underline{28}$						12 – – – – – – 12 — 12 — 12 – – – – – – 12 — 12 – – – – – – – – 12 — 12														
176							$\underline{28}$									$\underline{28}$ – – – – – – – – – – – – – – – – – – $\underline{28}$ – – – – – S_{28} – – – – – – – $\underline{28}$								
[illegible]	[illegible]																						
185	9	/	12 — 12 – – – – – 12						12–12 — 12 — 12 – – – – – – – 12 / 1 / 12 – – – – 12 – – – – – – 12 – – – – – – / [illegible]														
193	13*		13* — 13* — 13*						13* — 13*										13*		NT		

[illegible] failed [illegible] which [illegible] presented [illegible] que [illegible] prolonged [illegible] us to [illegible] que [illegible] shortened [illegible] que [illegible] a certain [illegible] in a [illegible] positive [illegible] (2) [illegible] of the [illegible] mar[illegible] 3, w[illegible] con[illegible] of t[illegible] time[illegible]

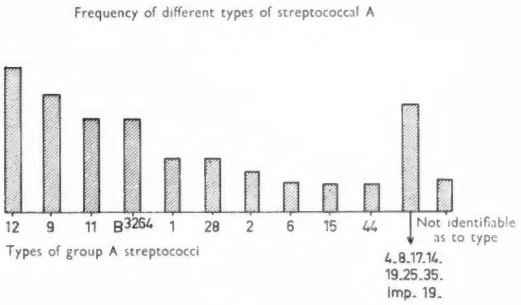

Fig. 7.

time, some even throughout the duration of the study (nos. 102, 136, 193). On the contrary, others, in the proportion of 1 to 3, while remaining carriers in the course of many swabbings, present frequent changes of type (nos. 10, 50, 52, 103, 116, 146 ...). (2) In the interval between two swabbings positive for the same type we are justified in assuming that the subject has remained a carrier of this strain, and that consequently all the swabbings in between should have been positive had our technique been perfect. Thus the gaps noted in prolonged carriers of the same type enable us to evaluate the accuracy of our technique of organism identification. In the present case, technique 3 was used, of which it may therefore be said that it failed in 34.5 % of cases. (3) These results have also enabled us to evaluate the frequency of the types of streptococcus A encountered (Fig. 7). Types 12, 9, 11 and B 3624 were the most frequent and were followed by 1, 28, 2. Finally, types 4, 6, 8, 14, 15, 17, 19, 25, 35, 44 and imp. 19 were seen occasionally.

VI—*Streptococcal sore throat*

In the whole child population observed over a period of 2 years only one case of streptococcal sore throat was seen (diagnosed clinically and bacteriologically). No case of scarlet fever was noted nor of any of the diseases considered as complications of streptococcal infections (rheumatic fever, acute hemorrhagic nephritis).

(Part II of this paper will be published in the next issue.)

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From the Department of Pediatrics, Harvard Medical School and the Children's Hospital, Boston

The Relationship Between Age and Fibrinolytic Activity of Serum

by ELIZABETH A. SAMARTZIS and CHARLES D. COOK

A number of studies have reported measurements of fibrinolysin in the serum of adults, both normal and abnormal (2, 3, 6, 10), pregnant women (7), and newborn infants (1, 7, 8), and in certain human tissues (5). The present study consists of measurements of the fibrinolytic activity in the sera of 64 infants and children ranging in age from birth to 15 years. These results are compared to values obtained in 15 normal adults.

Material and Methods

The 8 normal premature and 20 normal full-term infants were either born at the Boston Lying-in Hospital or transferred to the Children's Hospital within 12 hours of birth. In these infants, cord blood was used 23 times, venous 3 and capillary blood 2 times. The remaining studies in infants and children were done on serum obtained primarily from venous blood (85 %) but occasionally from capillary blood. The subjects were either attending the well-baby clinic or presented at the Medical Out-Patient Department with minor diagnostic or therapeutic problems. A few of the children were studied prior to elective surgical procedures.

Supported by a grant (H-3659) from the National Institutes of Health.

Active fibrinolysin, free or profibrinolysin, total profibrinolysin and fibrinolytic inhibitors were measured in the subjects' serum using the technique of Phillips & Skrodelis (6) which has been described in a report dealing with the relation between circulating fibrinolysins and the hyaline membrane syndrome of newborn infants (9).

Results

The results of the studies are shown in Table 1 and in Fig. 1. There is a definite tendency for the free profibrinolysin and the total profibrinolysin to increase with years. While not statistically significant, active fibrinolysin seems somewhat less in children over 1 year and in adults.

Discussion

Except as noted in the report of fibrinolytic levels in the hyaline membrane syndrome (4), no significance can presently be attached to the changing levels of fibrinolytic activity encountered during maturation. The purpose of the present report is merely to provide a background for the study of the relation of the fibrinolysins to various disease processes.

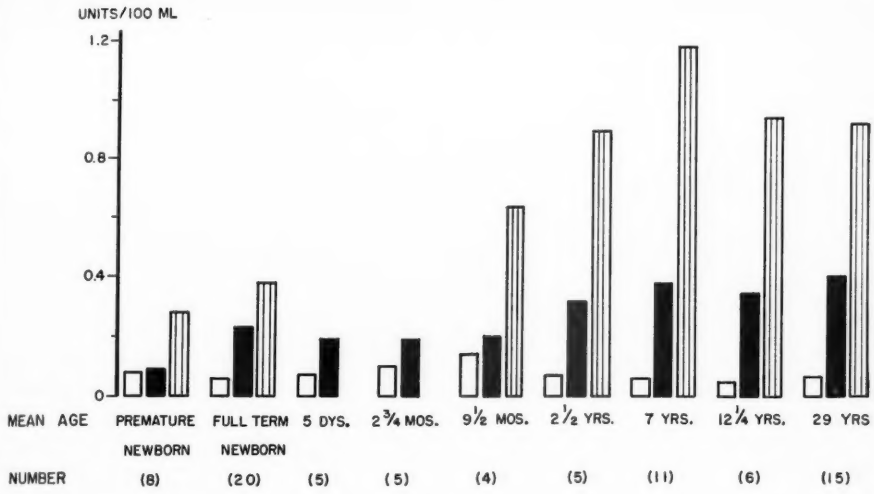

Fig. 1. Fibrinolytic activity in the serum of the 64 children and 15 adults studied. □ = active fibrinolysin, ■ = free profibrinolysin, and |||| = total profibrinolysin.

TABLE 1. *Fibrinolytic activity (units/100 ml) in infants, children, and adults.*

	Number of cases	Mean age	Active fibrinolysin	Free pro-fibrinolysin	Total pro-fibrinolysin	Inhibitors
Premature	8	N.B.*; G.A. = 34 wks.	.08±.04	.09±.07	.28±.08	.20±.08
Full term	20	N.B.*	.06±.02	.23±.18	.38±.27	.20±.24
4–7 days	5	5 days	.07±.04	.19±.08		
5 wks–4½ months	5	2¾ mos.	.10±.06	.19±.16		
7 months–12 months	4	9½ mos.	.14±.10	.20±.15	.64±.06	.44±.14
1 5/12–4 years	5	2½ yrs.	.07±.04	.32±.14	.90±.25	.58±.15
5–9 years	11	7 yrs.	.06±.04	.38±.09	1.19±.33	.81±.29
10–15 years	6	12¼ yrs.	.05±.05	.35±.11	.96±.35	.61±.42
Adults, 19–39 years	15	29 yrs.	.07±.06	.41±.23	.93±.34	.55±.32

* N.B. = Newborn; G.A. = Gestational age.

Summary

Measurements of active, free and total fibrinolysins in the serum of infants and children ranging in age from birth to 15 years are compared to adult levels. Older children and adults had higher values of free profibrinolysin and total fibrinolysin than newborn or young infants.

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Fibrinolytic Activity in the Serum of Infants with and without the Hyaline Membrane Syndrome

by ELIZABETH A. SAMARTZIS, C. D. COOK and A. J. RUDOLPH[1]

The pathogenesis of hyaline membrane disease in the newborn infant still remains obscure. The evidence presented by Gitlin & Craig (5) indicates that the membranes themselves are composed primarily of fibrin. The present investigation was undertaken at the suggestion of Dr. Gitlin to see if there was a relation between the level of circulating fibrinolytic activity in the serum of newborn infants and the presence or absence of hyaline membrane syndrome.

Material and Methods

Studies were performed on serum from 41 premature or diabetic mothers' infants (Table 1). In 27 of these the blood was obtained from the umbilical vein at birth; in the other 14, capillary or venous blood was drawn within 48 hours of birth. Twenty of the 41 infants had tachypnea and retraction and were diagnosed, by exclusion of other causes of respiratory distress, as probably having the hyaline membrane syndrome. In three of these infants who did not survive, the diagnosis was confirmed at autopsy. In 11 of the surviving infants x-rays were taken and 9 were consistent with the clinical diagnosis of probable hyaline membrane syndrome. In addition to this selected group of infants with a high incidence of respiratory distress, cord blood from 20 normal full-term infants was studied as well as venous blood from 15 normal adults, 21 normal mothers at term and 16 diabetic mothers at delivery. In order to see if venous and capillary bloods were comparable, the fibrinolytic activity was simultaneously measured in venous and capillary blood from 8 normal adults.

Fibrinolytic activity was determined by the spectrophotometric method of Phillips and Skrodelis (7) which measures the lysis of casein. One tenth ml of serum is incubated for two hours at 37°C with a 1 % casein solution and a phosphate buffer. The measurement is carried out in three tubes. In Tube I (the control) immediate inactivation is effected with trichloro-acetic acid. In Tube III profibrinolysin is activated with streptokinase. Following centrifugation the optical density of the supernatant fluid is measured at 280 mμ. The optical density of Tube I is subtracted from the optical density of Tubes II and III. The differences are then converted into units of fibrinolytic activity using a reference curve. The proteolytic activity in Tube II represents active fibrinolysin (plasmin), while the activity in Tube III represents the active fibrinolysin plus the activated enzyme derived from "free" profibrinolysin or plasminogen. The difference between the

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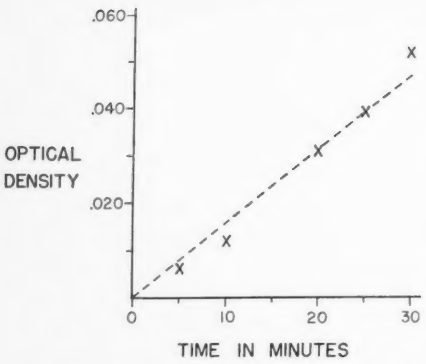

Fig. 1. Time curve obtained after incubation of 0.1 ml. enzyme preparation with 1 % casein solution for 5 to 30 minutes.

activity of Tube II and Tube III represents the amount of "free" profibrinolysin. However, inhibitors are present and in order to get total fibrinolysin and profibrinolysin the inhibitors are removed by the addition of acetic acid, refrigeration for 12–30 hours, and then centrifugation. The precipitate is redissolved in phosphate buffer and active and activated fibrinolysin determined as outlined above. Again the units of total active enzyme are subtracted from the activity measured after streptokinase activation in order to obtain the total profibrinolysin value. The difference between the "free" profibrinolysin and the total profibrinolysin provides an estimate of the amount of inhibitors present.

A reference curve is established using a standard preparation of fibrinolytic material.[1] A time curve for the lysis of casein is first prepared (Fig. 1). The units of enzyme activity per 100 ml are arbitrarily defined as shown in the equation.

$$\text{Units} = \frac{\text{Change in O.D.}}{(\text{ml of enzyme})\,(\text{time in minutes})} \times 100.$$

Having determined the activity of the preparation, a reference curve is made by incubating various quantities of the enzyme with the casein for a standard period of 2 hours. The results are shown in Fig. 2.

Although this technique followed that of Phillips & Skrodelis, the results in terms of units for comparable groups of patients were different. The differences are based on variation between lots of casein and "standard" lytic agents so that each laboratory tends to obtain different results. Hence, it is unfortunately necessary for each group of workers to establish control and patient values using, as was done in the present study, uniform reagent preparations throughout.

The measurements of fibrinolytic activity obtained both for a number of sera and for commercial fibrinolytic agents[2] using the above method were confirmed by the fibrin plate method of Astrup (1). That is, several sera with little or no casein lysis showed little or no fibrin plate lysis while sera with normal levels of casein lysis showed relatively larger areas (12×14 mm) of lysis on the fibrin plates. Similar correlations were obtained with the commercial preparations.

Results

The results of the studies on the sera are shown in Table 1 and in Fig. 3. The concentration of active fibrinolysin was essentially similar for all groups studied except the diabetic mothers who had significantly more than the full-term infants. Free profibrinolysin tended on the average to be significantly higher in pregnant women (both normal and diabetic) at the time of delivery than in the adult controls. Normal newborn infants had values for free profibrinolysin which averaged only 42 % of the normal mother's value a

[1] Plasmin ® was supplied by Merck, Sharp & Dohme Research Laboratories, West Point, Pennsylvania.

[2] Plasmin ® as well as Actase ®, supplied by the Ortho Research Laboratories, Raritan, New Jersey, were studied.

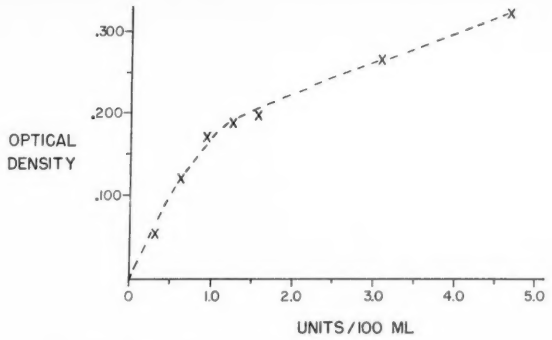

Fig. 2. Reference curve of fibrinolytic activity in units per 100 ml.

TABLE 1. *Fibrinolytic activity in Groups studied (units/100 ml)*

	Number of cases	Gestational age	Active fibrinolysin	Free profibrinolysin	Total profibrinolysin	Inhibitors
Full term	20	40	.06±.02	.23±.18	.38±.27	.20±.24
Premature	8	34(30–38)	.076±.037	.085±.072	.282±.075	.20±.08
<36 weeks	5	32(30–34)	.08	.08	.29	.17
>36 weeks	3	37(36–38)	.07	.09	.29	.20
Diabetic mothers' infants without respiratory distress	13	36(35–39)	.075±.06	.087±.05	.54±.47	.45±.47
Infants with respiratory distress*	20	36(34–39)	.075±.05	.070±.08	.298±.16	.22±.17
Infants with proven hyaline membranes	3	36(34–39)	.080±.046	.003±.006		
Normal adults	15		.07±0.06	.41±.23	.93±.34	.55±.32
Normal mothers at term delivery	21		.09±.14	.55±.18	1.30±.45	.75±.40
Diabetic mothers at delivery	16		.10±.07	.57±.18	1.68±.71	1.06±.55

* Includes the 3 with proven membranes at autopsy. Ten were premature and 10 were premature and born to diabetic mothers. Eleven specimens were obtained from the umbilical cord and nine from venous and capillary blood. There was no apparent difference in the results which could be related to the source of the material.

significant difference. Premature infants had significantly less free profibrinolysin than full-term infants. No trend from marked to moderate prematurity was evident; this was at least partly because of the variation in values and the small number of cases in each group. The diabetic mothers' infants had approximately the same free profibrinolytic activity in their sera as non-diabetic mothers' infants of approximately comparable gestational ages. The 20 infants with clinical evidence of the hyaline membrane syndrome had, on the average, slightly but not significantly less free profibrinolysin than normal premature infants or healthy infants of diabetic mothers. However, the three of these infants who died and were proved

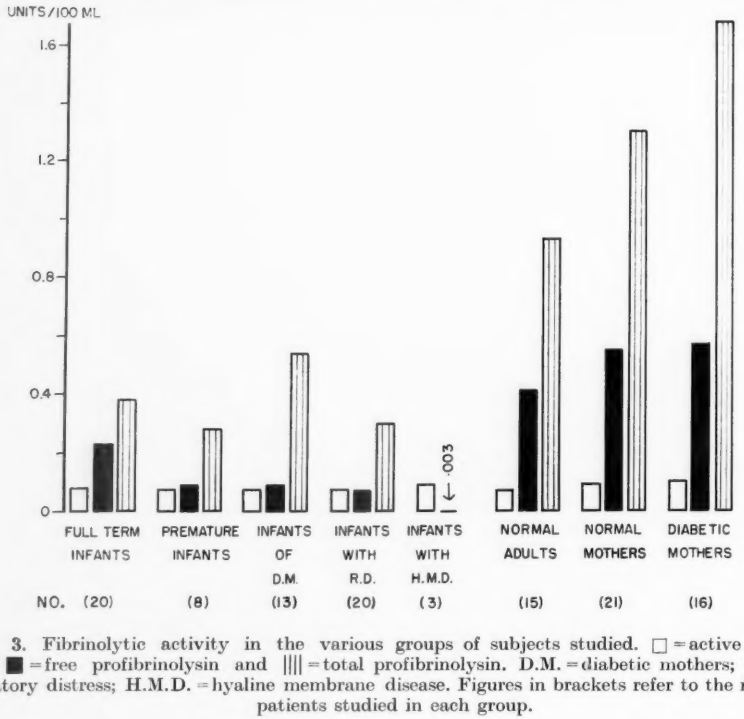

Fig. 3. Fibrinolytic activity in the various groups of subjects studied. □ = active fibrinolysin; ■ = free profibrinolysin and |||| = total profibrinolysin. D.M. = diabetic mothers; R.D. = respiratory distress; H.M.D. = hyaline membrane disease. Figures in brackets refer to the number of patients studied in each group.

to have hyaline membranes at autopsy averaged significantly less free profibrinolysin than any of the other groups. In these three infants the blood for analysis was obtained before the infant became moribund.

In all the groups of infants in whom total profibrinolysin activity was measured it was found to be significantly lower than in adults; and both normal mothers and those with diabetes had values significantly higher than both the infants and non-pregnant adults. There were, in general, less inhibitors present in infants than adults, although both the diabetic mothers and the diabetic infants tended to have higher levels than the other

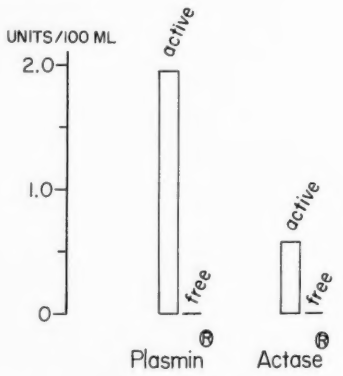

Fig. 4. Measurement of active and free fibr o-lytic activity in concentrated solutions of co commercial preparations. According to the m a-facturers' specifications 3200 "units"/ml of ne Plasmin (R) and 12,500 "units"/ml of Ac se (R) were used.

TABLE 2. *Comparison of fibrinolytic activity in the venous versus the capillary blood of 8 normal adults.*

No. of subjects	Active		Free	
	venous	capillary	venous	capillary
8	.084±.06*	.025±.05*	.47±.10*	.46±.16*

* No significant difference was found between venous and capillary determinations.

mothers and infants respectively. No correlation was found between an individual infant's level of fibrinolytic activity and that of the mother.

A close relation between seven out of eight simultaneous measurements of fibrinolytic activity in venous and capillary blood of adults was found, particularly for free profibrinolysin (Table 2) and it was, therefore, assumed that cord and venous blood could be compared to capillary blood in respect to fibrinolysin.

Discussion

Quie & Wannamaker (9) have reported a deficiency in the profibrinolysin activity of the serum of newborn infants when compared to adult values. Studies by Phillips & Skrodelis (8) have demonstrated the lower levels of fibrinolytic activity in the serum of premature infants compared to full-term infants. The present studies confirm the relation between circulating fibrinolytic activity at the time of birth and the presence or absence of prematurity. They also suggest that there is no relation between the presence of the respiratory distress syndrome (i.e. the hyaline membrane syndrome diagnosed on a clinical basis) and the levels of fibrinolytic activity. However, the studies do suggest that if the respiratory distress is severe enough to lead to respiratory failure and death, the profibrinolysin levels may be found to be depressed. Unfortunately in the present series, measurements of fibrinolytic activity were made in only three infants with this most severe form of the syndrome and in all three cases the measurements were made not on cord blood, but on capillary or venous blood obtained during the first 24 hours of life. A comparison between the cord blood and venous and capillary blood measurements and serial determinations in four normal newborn infants showed no trend toward decreasing amounts of fibrinolytic activity with increasing post-partum age; thus apparently the decreased or absent fibrinolytic activity in the sera of the infants who succumbed to the disease was a significant finding. Although it is possible that these three infants had low fibrinolytic activity at birth, it seems equally probable that, as their disease progressed, their fibrinolysins decreased because they were used in lung tissue. These studies are in accordance with those of Lieberman (6) who reported absent fibrinolytic activity in the lung tissue of infants dying with hyaline membranes. The most important question remaining is whether the deficiency in fibrinolysin (shown in the sera in the present study and tissue in Lieberman's study) is a primary factor in the pathogenesis of the membranes or whether it is secondary to utilization of fibrinolysin following membrane formation. In any case, it is possible that the low fibrinolytic activity encountered in prematures is at least a contributing factor. As suggested by Avery & Mead (2), the most important deficiency may be

the absence of a substance which reduces the surface tension of the air sacs.

Regardless of whether or not a serum or tissue deficiency in fibrinolytic enzyme is a primary factor, the possibility of lysis of existing membranes with various therapeutic agents has been suggested by a number of authors (3, 4, 6). With this in mind, two of the available commercial preparations were tested *in vitro* and in experimental animals. The results are shown in Fig. 4. As can be seen, the amount of active lytic enzyme in the concentrated preparation, even in the most potent, is only equal to approximately 20 times the active and 5 times the free profibrinolytic activity of adult sera. If 50 ml of this could be given and were distributed throughout the body fluid compartment of a newborn 2 kg infant, only approximately 0.07 units of fibrinolytic activity would be added per 100 ml. It seems unreasonable to suppose that this would produce much lysis even if it reached the membranes. When 6.5 ml of plasmin (R) (concentration = 3200 "units"[1]/ml or 1.95 units/100 ml by our method) or 2 ml of actase (R) (concentration = 12,500 "units"[1]/ml or .58 units by our method) were given to two 4 kg rabbits, no detectable change in fibrinolytic activity of the sera could be noted at either 2 or 5 hours. Rabbits have a low fibrinolytic and profibrinolytic titre (0 and .07 units/100 ml respectively) and hence the streptokinase-streptodornase was ineffective and the fibrinolytic material administered was apparently too little to change measurably the activity of the serum.

Much of the reported activity of the commercial preparations is apparently due to the activation of the patient's own profibrinolysin by the streptokinase-streptodornase which they contain (10). Although this reaction may be possible in the newborn infant, the low amount of proenzyme available in the circulation for conversion raises doubts about such an occurrence. However, when non-pyrogenic preparations are available, their effect on the newborn infant's fibrinolytic system will need evaluation.

The administration of fibrinolytic enzymes by aerosol has also been tried (4) but to date without convincing results. Again, the problem of concentration of a sufficiently potent enzyme at the proper site remains. The work of Craig, Fenton & Gitlin (3) indicated that high concentrations of fibrinolytic enzyme preparations had to be incubated with lung tissue slices for a number of hours before dissolution of the membranes occurred. In this connection, it was found that amniotic fluid from two normal pregnancies showed no measurable fibrinolytic activity and hence apparently does not function to prevent membrane formation. More controlled trials of aerosols are indicated but will be difficult to evaluate except in experimental animals.

Summary

(1) The fibrinolytic activity of the serum of normal full-term and premature infants has been compared to that of diabetic mothers' infants and to those with the hyaline membrane syndrome.

(2) The amount of active fibrinolytic enzyme was the same in all groups of infants. The amount of free profibrinolysin (plasminogen) was related to the prese

[1] These are the manufacturers' units.

or absence of prematurity, being lower in those born prematurely. There was no significant relationship between circulating profibrinolysin and the presence or absence of respiratory distress. However, three infants dying with proven hyaline membranes did have significantly lower levels of circulating profibrinolysin. It was postulated that this deficiency might be a contributory and/or secondary factor in the membrane formation.

(3) The mothers, both normal and diabetic, have more free profibrinolysin and more inhibitors than the infants.

(4) Two commercial preparations of fibrinolytic enzymes were found to contain relatively small amounts of fibrinolytic activity.

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Blood Oxygen Tension Determination for the Recognition of Hypoxia in Newborn Babies and Older Infants[1]

by J. WENNER

Clinical signs of hypoxia, such as cyanosis, unconsciousness and respiratory embarrassment are not very reliable as indicators of the degree of oxygen lack.

For example it is well known that cyanosis, usually considered the most important symptom, may be absent in severe oxygen lack in an anaemic patient. On the other hand, cyanosis may be present due to blood stasis in the skin without any hypoxia of more vital organs. Other clinical symptoms are not specific for hypoxia.

Quantitative statements about the degree of hypoxia must be based on analysis of the blood gases. Analysis of arterial blood gases will demonstrate hypoxia only when there is deficient oxygen saturation in the lungs or a right-to-left-shunt. However, in the more common ischaemic hypoxia the arterial blood gases are normal. For this reason knowledge of the values of the venous blood gases are more important.

This fact can be demonstrated by Krogh's schematic representation of the oxygen supply to the tissues (6). Figure 1 shows a cylinder of tissue, which is supplied by one capillary from which the oxygen diffusion into the tissue takes place. The lower part of the figure shows a section through the cylinder. It is the difference between the oxygen concentration of the blood plasma and of the tissue which is reponsible for the diffusion rate.

The oxygen concentration in the blood plasma is determined by the oxygen tension of the blood. At the venous end of the capillary the lowest tension is obtained. Therefore this last part of the tissue cylinder is less protected in all forms of hypoxia. This is the case if there is already insufficient oxygen saturation in the arterial blood or if this oxygen lack develops during the blood flow in the capillaries in cases of ischaemia or anaemia. The oxygen tension in the venous blood of any organ is an adequate measure of the oxygen supply in the less protected end-portions of the tissue cylinders in the organ. Therefore it can be stated that the venous oxygen tension gives a measure of the degree of hypoxia due to any cau[illegible].

[1] Presented in part at the IX. Intern. Congress of Pediatrics, July 1959, Montreal, Canada.

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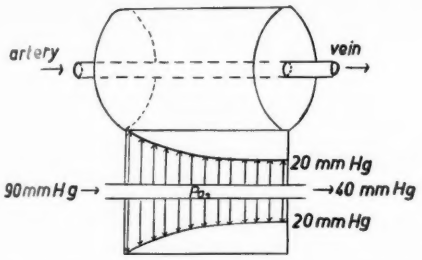

Fig. 1. Krogh's diagram of the oxygen supply to tissue cylinders (6). Each cylinder is supplied by one capillary from which the oxygen diffusion into the tissue takes place. The lower part of the figure shows a section through the cylinder. The oxygen tension at the venous end of the capillary is an adequate measure of the oxygen supply to the less protected end-portions of the tissue cylinder.

From the clinical point of view it is oxygen lack in the brain which is of special interest. In investigating the oxygen supply of the brain in infancy, it is relatively simple to withdraw cerebral venous blood from the superior longitudinal sinus.

To obtain an idea of the normal oxygen supply of the brain in infancy the percentage oxygen saturation, the oxygen tension and the pH of cerebral venous blood as well as the arterio-venous oxygen difference were determined in 67 healthy infants. In addition oxygen tension was determined in 40 sick but afebrile infants in whom oygen lack seemed likely to be present.

Methods

The oxygen saturation was calculated from the oxygen content of the venous blood and the oxygen capacity; both estimated in a van Slyke manometric apparatus.

The oxygen tension of the venus blood was measured by the potentiometric method with a mercury electrode devised by Bartels (1). For this method 4 ml of blood were needed. Recently the polarographic method using a Clark platinum electrode was applied to the oxygen tension determination (9) and less than 1 ml of blood was required.

For the determination of the arterio-venous oxygen differences in healthy infants the arterial oxygen content was calculated for an assumed arterial oxygen saturation of 95 %. The venous oxygen content was measured as mentioned above.

Results and Discussion

Oxygen lack has usually been investigated by determination of the oxygen saturation of blood. This method may be used in adults when pH is known, because of the correlation between oxygen saturation and oxygen tension of blood given by the oxygen dissociation curve of the blood. In infants this method does not give accurate information as the following figures demonstrate.

In Fig. 2 the percentage of oxygen saturation of cerebral venous blood in healthy infants is plotted on the ordinate and the infants' ages on the abscissa. The lines represent the mean values and the corresponding standard deviations. The differences between the mean values are statistically significant on the basis of Student's t test in this and the following figures.

It is shown in Fig. 2 that the oxygen saturation during the first 3 months of life is lower than in older infants; it is also shown that the saturation in the first 50 days of life is higher than in the subsequent 50 days.

The higher oxygen saturation in the first 50 days of life might lead one to conclude that the oxygen supply of the brain is better at this age than in the following

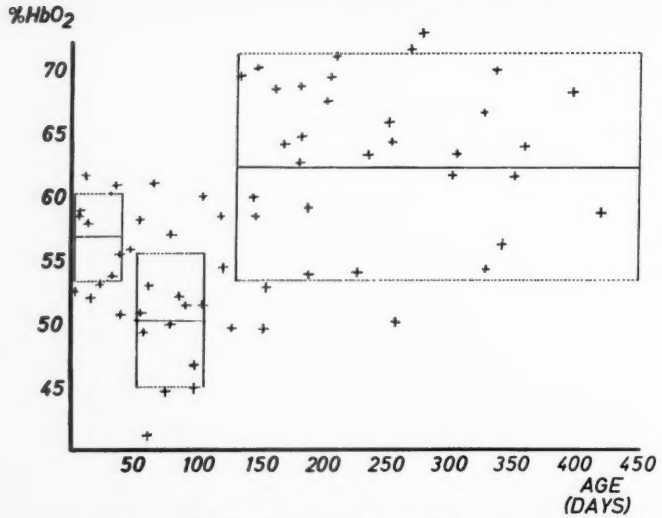

Fig. 2. Oxygen saturation (% HbO_2) of cerebral venous blood (superior longitudinal sinus) in 67 healthy infants. The infants' ages are plotted on the abscissa. The lines represent mean values and corresponding standard deviations. The differences between the mean values are statistically significant.

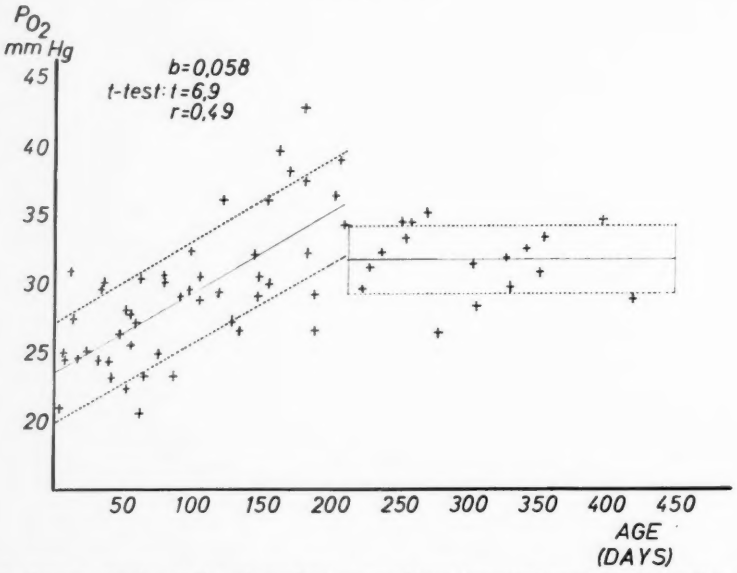

Fig. 3. Oxygen tension (PO_2) of cerebral venous blood (superior longitudinal sinus) in 67 healthy infants. The infants' ages are plotted on the abscissa. The regression line, the mean value and corresponding standard deviations are shown. During the first six months of life the PO_2 increases gradually. b = correlation coefficient. b is statistically significant (t test). r = regression coefficient.

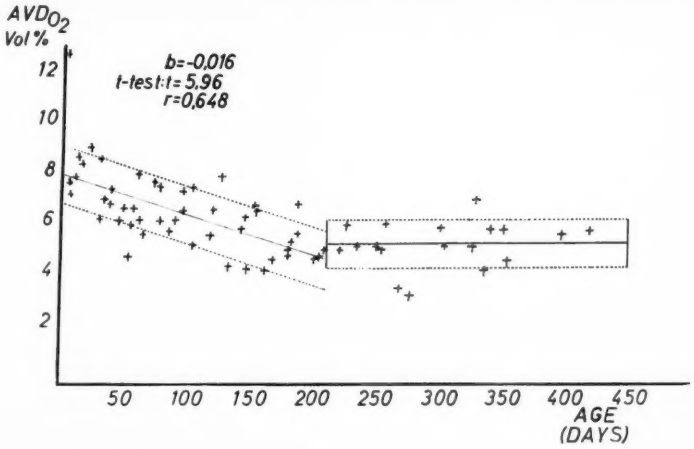

Fig. 4. Oxygen content differences (Vol %) between arterial blood and cerebral venous blood ($AVDo_2$) in 67 healthy infants. The infants' ages are plotted on the abscissa. During the first six months of life the AVD decreases gradually. The correlation coefficient "b" is statistically significant (t test).

50 days. This conclusion, however, would be wrong as shown by the oxygen tensions of the cerebral venous blood (Fig. 3).

The lowest oxygen tension is found in the youngest infants and it increases gradually during the first six months. This means that it is the newborn and youngest infants who have the poorest oxygen supply to the brain.

The oxygen tension in the cerebral venous blood is normally lower in infants than in adults, in whom values of about 35 mm Hg are found (5, 8). Opitz & Schneider state that in adults an oxygen tension of about 20 mm Hg is the critical level below which unconsciousness occurs (8). In healthy newborns oxygen tension close to 20 mm Hg were found.

The lower oxygen supply to the brain in younger infants can also be demonstrated by the arterio-venous oxygen differences; the difference is highest in the youngest infants (Fig. 4).

The discordant changes in oxygen saturation and oxygen tension in the first 3 months can not be explained by changes in blood pH, since the pH was even lower in the youngest than in the older infants.

The discrepancy between oxygen tension and saturation is explained by the shift in the blood oxygen dissociation curve which has been shown to take place during the first months of life by Beer *et al.* (3). There is a statistical correlation between the shift of the dissociation curve and the reduction in the amount of foetal haemoglobin in the first months of life. However, this does not necessarily imply a causal relation; there is also a correlation between the shift in the dissociation curve and changes in the shape of the red blood cells (2).

Fig. 5 shows the part of the dissociation curve related to the range of oxygen saturation concerned in this investigation (3). The middle curve applies to adults,

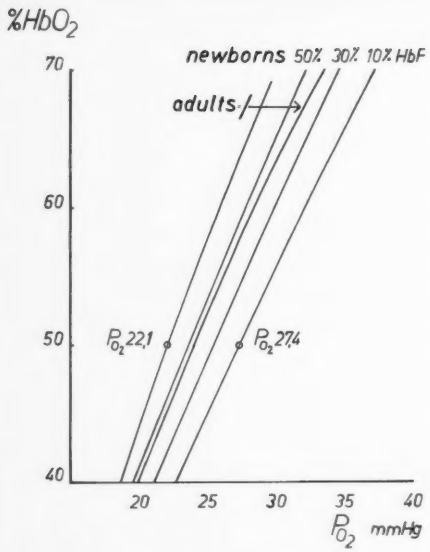

Fig. 5. The shift of the oxygen dissociation curve of blood in infancy correlated with the reduction in foetal haemoglobin. The figure shows parts of oxygen dissociation curves (Beer, Doll & Wenner (3)). The blood pH was 7.4, the temperature 37°C. The curve in the middle applies to adults, that on the left side to newborn babies with high foetal haemoglobin concentrations and that on the right to older infants with only 10 % Hb F. At 50 % oxygen saturation PO_2 may vary more than 5 mm Hg.

that on the left side to newborn babies with high foetal haemoglobin concentration and that on the right to older infants with only 10 % foetal haemoglobin. The oxygen dissociation curve of older infants is located more to the right of the standard dissociation curve of adults than has been shown in the investigations of Morse, Cassels & Holder (7).

At an oxygen saturation of 50 % the oxygen tension in infancy may vary more than 5 mm Hg (Fig. 5). Therefore, in order to show with certainty the degree of oxygen lack, direct measurement of venous oxygen tension is necessary.

In Fig. 6 the mean values and standard deviations of cerebral venous oxygen tension in healthy infants (Fig. 3) are compared with values obtained in infants suffering from various diseases, viz. congenital heart failure with and without cyanosis, anaemia with haemoglobin below 40 %, diarrhoea with dehydration, subdural effusion following meningitis and a few cases of hydrocephalus.

In younger sick infants oxygen tensions as low as 10 mm Hg were found without any signs of unconsciousness. In one case the tension was only 7 mm Hg; the infant was unconscious and died a few days later.

In cases of heart failure with cyanosis—especially in older infants—hypoxia of the brain may be missed. This may be the result of an increase in the rate of cerebral blood flow as has been demonstrated in adults by Gottstein *et al.* (4).

Figure 6 includes 16 oxygen tension values in 12 infants with subdural effusion, investigated by Wenner & Koch (10). These oxygen tensions are within the normal range and this means that there was no *general* ischaemic hypoxia of the brain in these children. The size of the subdural effusion in the infants was between 20 and 100 ml, but there was no marked increase in intracranial pressure. This may be explained by the softness of the infant's cranium and the open sutures.

This study is presented to demonstrate that estimation of cerebral venous blood oxygen tension is of value in determining the presence or absence of cerebral hypoxia in ill newborn babies and older infants.

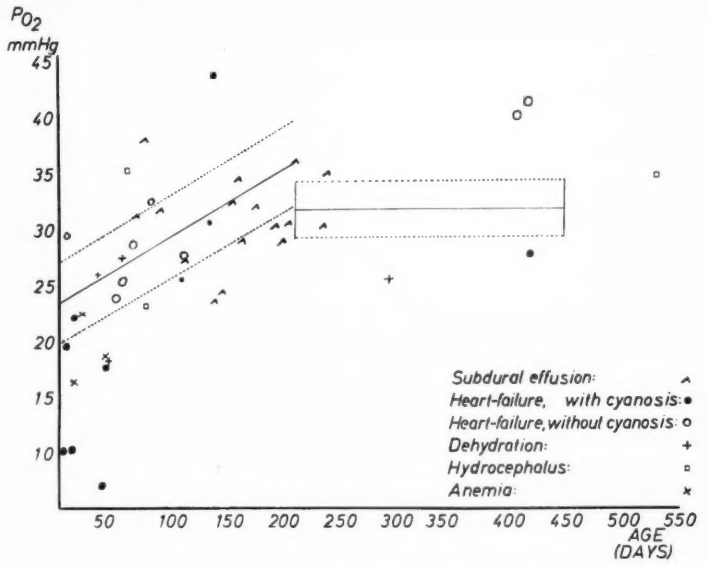

Fig. 6. Oxygen tension of cerebral venous blood in sick children. The lines represent mean values and corresponding standard deviations of oxygen tensions in healthy infants (see Fig. 3). These normal values are drawn for the purpose of comparison with single values obtained in infants with various diseases in whom oxygen lack seemed likely to be present. The infants' ages are plotted on the abscissa.

Summary

Estimations of oxygen tensions, saturations and arterio-venous oxygen differences of cerebral venous blood withdrawn from the superior longitudinal sinus have been carried out in 67 healthy infants and 40 infants with various diseases.

It has been shown that direct measurement of the oxygen tension is necessary in order to assess cerebral oxygen lack in infancy.

It was found that the oxygen tension of the cerebral venous blood of healthy infants is lower than the accepted values for adults. The oxygen tension was found to be lower in newborn than in older infants.

An oxygen tension of 20 mm Hg is said to be the critical level below which adults become unconscious; the investigations here reported show that this is not true for infants. Even in healthy young infants oxygen tensions were found close to 20 mm Hg. Moreover, unconsciousness was not observed in hypoxic newborns in whom cerebral venous oxygen tension was as low as 10 mm Hg.

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Multiple Occurrence of Atrial Septal Defect in a Family

by PER ZETTERQVIST

Reports of familial incidence of congenital heart disease—identical or not—are relatively rare, and include for the most part not more than 2 members of the same family, as a rule siblings or a child and a near relative of one of the parents. Atrial septal defect (ASD), however, has been diagnosed at times in 3 or 4 members of the same family, and several times it has been found in parent-child constellations. For these reasons, the possibility of a real inheritance factor has been brought up. The family which will be presented here gives further support to this theory and has so many cases of certain or probable atrial septal defect that it can in addition serve to illustrate the natural history of the disease as well as some aspects of its symptomatology and diagnosis.

In Fig. 1 is shown the pedigree, which illustrates all certain or suspected cases of ASD in the family together with siblings and descendants. *Generation I* is represented by a woman born in 1821. She was reportedly feeble, and she died at the age of 60. There is no real evidence of heart disease from what is known about her, and she was never examined by a doctor. She had 5 children, presented in the pedigree as *Generation II*. Of these the oldest daughter died at the age of 6 of an heart attack after previously having been healthy and having had 5 children. The second sister also seems to have been healthy, as she married after the age of 40, later had 4 children, and reached an age of more than 85 years. Her brother II.3, born in 1858, had a known heart disease from his youth on. He was a farmer and very active but avoided heavy physical work. He died at the age of 81 years. Sister II.4, born in 1860, also had a known heart lesion from youth. She is described as active but not very strong. She remained unmarried and took care of the household until 1898, when she could not work any longer because of increasing dyspnea and tiredness on effort. Because of an eye complaint she was examined at that time by a doctor. To his complete suprise she lived another 12 years. The cause of death according to the parish registry was organic heart disease. The youngest child in the siblingship, II.5, died at the age of 6½ months of unknown reasons.—In none of these cases was autopsy performed.

The members of *Generation III* are all children of II.3. About them information is more detailed. Only in the oldest of the siblings, a man born in 1899, can ASD be excluded, as he has no symptoms, normal physical heart findings and a normal ECG. Among the remaining 5 cases the diagnosis is certain or highly probable in 4, and in one case it seems rather possible. The essential data are the following:

III.2, a farmer's wife born in 1901, showed, from early childhood, signs of a heart lesion but lived an essentially normal life until the age of 44, when she began to have ankle

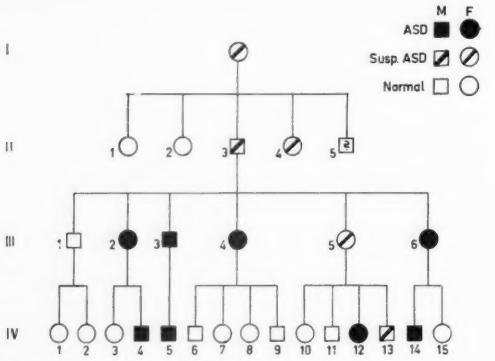

Fig. 1. Pedigree of the family.

edema. In spite of treatment with digitalis and diuretics she became worse with increasing edema and severe generalized cyanosis. Six months later she got pneumonia. After a few days she had an arterial embolism to one arm and suddenly died in an acute cerebral vascular accident. Autopsy was not performed.

III.3, a farmer born in 1904, has from the age of 30 noticed slowly decreasing physical working capacity but had in 1959 still no definite heart symptoms. At physical examination (Aug. 1959) his general condition was found to be good with no signs of heart failure at rest. Inspection of the precordium revealed nothing abnormal. At palpation the parasternal pulsations were not found to be definitely increased, and no real parasternal lift was felt (well-developed thoracic wall). However, the apex beat was not distinctly palpable, and at auscultation a rather harsh early systolic murmur of Grade 3 intensity and a widely split second sound were heard over the pulmonary region. ECG and roentgen findings, obtained in 1944 and then thought to be due to a chronic myocarditis, virtually confirm the diagnosis of an ASD of the secundum type.

III.4 is a farmer's wife born in 1906. Heart disease has long been known, but the nature of it was uncertain. She has been feeble but has had the cleverness to organize her work with the aid of household machines, thus avoiding physical exertion. She has had 4 children, the last one born in 1953. No obvious heart symptoms were noticed until 1958, when she began to have ankle edema and associated with this slight cyanosis of the legs. With digitalis and chlorothiazide these symptoms have vanished, but the patient becomes short of breath on moderate exertion and has in addition noticed palpitations, especially when she gets nervous. At physical examination there were signs of marked right ventricular dilatation, and the systolic ejection murmur over the pulmonary region was definitely harsh though not very strong (Grade 1–2). ECG showed a pattern of incomplete right bundle branch block with right axis deviation and clockwise rotation of the frontal vector. In addition there was atrial fibrillation and, on moderate exercise, frequent ventricular extrasystoles of multifocal origin. Roentgen examination showed the picture of a left to right shunt at the atrial level with a heart volume of 1700 ml corresponding to 1000 ml per sqm body surface area. Catheterization and angiocardiography in 1960 have confirmed the diagnosis of an ASD. The flow in the pulmonary circuit was calculated to be 13.4 and in the systemic circuit 4.7 liters per minute; the ratio between them is thus about 3:1 (at rest, ventricular rate 64 beats per minute). Operation is planned.

III.5, a farmer's wife born in 1909, is completely free of heart symptoms and in very good condition in spite of obesity. Because of this obesity the physical heart findings were difficult to assess, but they were not considered pathological. ECG, however, showed rather advanced changes as encountered in ASD and similar to those found in Case III.3. In spite of a negative roentgen examination the patient is still suspected of having a small ASD.

III.6, a pharmacist born in 1911, is the mother of two children, the last one born in 1950. Since childhood she has been somewhat feeble and in addition short of breath with moderate exertion, and after having been ill with influenza in 1957 she has several times had protracted bronchitis with increased dyspnoea. Examination in October 1959 including catheterization and angiocardiography revealed a large ASD of the secundum type associated with mitral insufficiency due to relative dilatation of the mitral ring without malformation of the cusps. The relative heart volume was 900 ml per sqm body surface area, and the flows through the pulmonary and systemic circuits 34 and 4.1 liters per minute, respectively. At operation performed in November 1959 at the Thoracic Clinic, Karolinska Sjukhuset, with extracorporeal circulation and moderate hypothermia, both malformations were corrected.

Generation IV consists of 15 members. Two of them, IV.4 and IV.14, on routine examinations were found to have signs of heart diease. At catheterization and angiocardiography, an ASD of the secundum type was diagnosed in both. IV.4, a boy born in 1938, was operated on in 1954, but in the Case IV.14, a boy born in 1948, operation was not considered to be indicated, as the pulmonary/systemic flow ratio was shown to be only 1.5:1. In 1959 the remaining members of this generation, all being normally developed and free of symptoms, were examined physically and electrocardiographically. In these cases nothing of definite pathological significance was found at the physical examination, but in some of them the systolic ejection murmur over the pulmonary area was well audible (Grade 2), and in Case IV.5 it was also a little harsh. In 3 cases ECG showed incomplete right bundle branch block with right axis deviation. In these roentgen examination was performed. Two of them, IV.5 and IV. 12, showed the picture of a left to right shunt at the atrial level with obvious heart enlargement. In the third case, IV.13, the roentgenological findings were not of pathological significance.

As can be seen from Fig. 1, eight members of Generation II and IV have been regarded as having atrial septal defect, even if the diagnosis has not been anatomically demonstrated in more than four (III.4, III.6, IV.4, and IV.14). In Case III.2 the history of classical "cyanose tardive" and death, most probably due to paradoxical embolism, is quite typical. In Cases III.5, IV.5 and IV.12, the physical findings together with ECG and roentgen examination speak strongly in favour of the same diagnosis. Among the cases listed as "suspected ASD" this suspicion has been based on the ECG findings in two and on the history in the others. (It should be noted, that in the individual cases in Generation I and II the diagnosis is only presumptive.)—In all definite or suspected cases which were investigated, the ECG pattern has been that characteristic of an ASD of the secundum type, i.e. incomplete right bundle branch block with right axis deviation and clockwise rotation of the frontal vector. In all anatomically verifed cases the defect has been found to be secundum type. In two patients associated malformations were found. Case III.6 had a mitral insufficiency secondary to relative dilatation of the mitral ring, and Case IV.4 had bilateral superior caval veins, an anomaly of no hemodynamic importance.

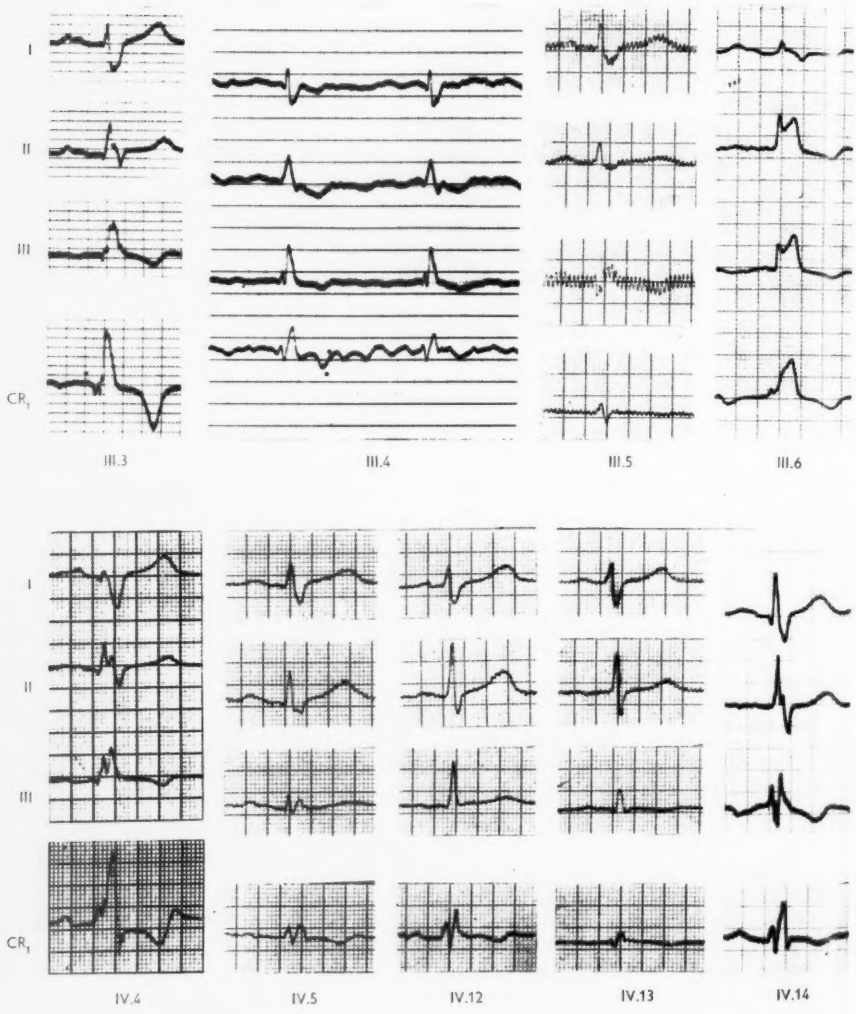

Fig. 2. Electrocardiographic findings. In all definite or suspected cases of ASD examined, the ECG pattern is that of an incomplete right bundle branch block with right axis deviation and clockwise rotation of the frontal vector. Note rS or RS complex with broad and deep S wave in lead I and rsr' or rsR' complexes in the right precordial lead CR_1. It is difficult to judge, if right ventricular hypertrophy exists in addition, but it seems likely in Cases III.3, III.6, IV.4 and IV.14, where the R' in lead CR_1 is rather large. Atrial fibrillation is present in Case III.4. (The ST changes in this case are probably due to digitalis therapy.)

Fig. 3. Roentgenological findings (frontal view) in the same cases as presented in Fig. 2. Gross enlargement of the heart is seen in cases III.3, III.4, and III.6, while it is moderate in Cases IV.4, IV.5, IV.12, and IV.14. The enlargement is due to dilatation of the right atrium and right ventricle. The pulmonary artery is widened and the vascularity of the lungs increased because of the shunt.—In Cases III.5 and IV.13, the roentgenological findings are normal.

Discussion

Genetic factors have long been suspected of playing some part in the etiology of congenital heart disease (CHD). Thus, an increased frequency of cardiac defects has been found among relatives of patients with CHD. In subsequent siblings of a child with CHD, the risk of being born with a cardiac defect has been calculated to be about 2 % (7), while the incidence for the general population is slightly less than 1 % (4). Extensive studies have shown that the relative importance of the genetic factors varies with different types of lesions (6). Definite examples of a mendelian mode of inheritance have been difficult to demonstrate. Families with more than two known cases are rare. Studies of the incidence of parental consanguinity have given only a weak support to the theory of recessive inheritance, and parent–child constellations pointing to a dominant mode of transmission are uncommon. This could in part be due to the natural history of the disease. In most types of severe CHD the course is such that the patients do not reach adulthood, or if they do, they do not have children because of their symptoms. In such cases it is not possible to demonstrate or exclude the existence of an inheritance factor. When identical CHD has been found in parent–child constellations, the lesion has also in most cases been of a type known to run a mild course during the first two or three decades. Among the latter group are some families with ASD (1, 2, 3, 4, 5, 8). This condition also seems to be the one that has been most often observed in more than 2 members of the same family. In two cases, ASD has been reported to occur in one parent and 3 children (out of 8 and 10 siblings, respectively) (5, 8). Davidsen, who reported one of these families, suggested the possibility of a dominant disease precursor. Judging from the otherwise normal family history, it may be that there was a mutation in one woman who transmitted the tendency to 3 children. Further support for the hypothesis of a dominant hereditary transmission may be present in one family reported by Campbell (2). In that case, ASD was found in one girl, her mother and maternal grandmother. A dominant hereditary transmission might also be operative in the family presented. If the "susp. ASD" characterized cases could be regarded as definite, this family could well satisfy the criteria for such a hereditary mode of transmission. Every affected person has an affected parent, all children of healthy individuals are healthy, and the incidence of ASD in children of affected persons is 12 out of 24, or 50 %.

Summary

A report is given of the multiple occurrence in a family of atrial septal defect of the secundum type. The pedigree suggests a dominant mode of inheritance, where 4 generations show 8 certain and 5 probable or suspected cases. In previous reports of identical congenital heart disease in parent–child constellations or in more than 2 cases in the same family atrial septal defect is commonly found. This might in part be due to a particular selective mode of inheritance. On the other hand, the fact that atrial septal defect is often compatible with a relatively normal life, at least concerning the first 3 decades, may also account for the appearance oftener than other more severe lesions.

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CASE REPORT

Generalised Oedema of Obscure Origin

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When there is no cardiac or renal cause for chronic generalised oedema, and there is no hypoproteinaemia, only detailed and complex investigations can be expected to reveal the cause of the condition. Below is a description of a case referred to us on account of generalised oedema of many years' duration.

Case Report

An otherwise healthy boy was referred to the Outpatient Department at the age of 12 years on account of oedema of the face, hands and ankles. The puffiness of the hands was noticed by his parents in infancy, and had persisted from that time. They said that his ankles had been swollen at night "for many years". The eyelids had been puffy every day for 8 years, apparently following an attack of orbital cellulitis, for which he had been treated in this hospital. (It was obvious on referring to the notes that this diagnosis was correct. Coagulase positive staphylococci were cultured from the pus which was evacuated.)

There were no other symptoms. The boy was a cross-country runner at his grammar school, and there had never been any symptoms suggestive of cardiac or renal disease. The diet had been reasonable in quality and quantity. There was no history of previous illnesses. There was no family history of oedema, heart disease, kidney disease or chronic diarrhoea. There were no other children.

On examination the only abnormal features were oedema and a trivial developmental anomaly of the toenails (Fig. 1–3). The oedema affected the lower limbs up to the midcalf, both upper limbs up to the forearms, and the face and eyelids. The oedematous tissues pitted readily. There was no detectable oedema over the sacrum or elsewhere, and there were no signs of serous effusions. His weight was slightly below the average for his age. The blood pressure was normal (and remained so on repeated examinations), and there was no detectable abnormality of the cardiovascular system. The jugular venous pressure was normal. The urine was normal chemically and microscopically. A clinical diagnosis of idiopathic hypoproteinaemia ("exudative enteropathy") was made, and he was admitted for investigation. This diagnosis was proved to be incorrect when it was found that the serum protein concentration was normal.

Investigations

These will be given in tabular fashion.

Urine (as already stated)—consistently normal chemically and microscopically.

Concentration and dilution test normal.

Intravenous pyelogram normal.

Blood urea (6 estimations): Mean 30 mg %. Range 20 to 65 mg %. (The latter was the only high reading.)

Serum proteins—(9 estimations): Total

Fig. 1. Oedema of face.

mean 6.6 g %. Range 5.2 to 8.0 g %. Albumin, mean 3.6 g %. Range 2.8 to 4.6 g %. Globulin, mean 2.9 g %. Range 2.0 to 4.2 g %. Fractionation—one specimen normal: some increase of alpha 2 and gamma globulin in another.

ECG normal.

Liver function tests normal: Serum alkaline phosphatase (mean of 3 readings) 17 units, Jenner & Kay. Serum bilirubin (3 readings) 0.5 mg %. SGP transaminase 44 units. SGO transaminase 10 units. Thymol turbidity (mean of 2 readings) 4.7 units. Zinc turbidity (mean of 2 readings) 26.5 units. Prothrombin time 12 seconds. Bromsulphthalein absorption test (5 mg/kg given): 100 per cent dye present in 6 minutes, 2.9 per cent in 30 minutes and 1.5 per cent in 60 minutes.

Nitrogen balance positive. (72 hour nitrogen balance on a daily intake of 11.3 g of nitrogen showed a positive balance of 0.37 g per day.)

Fat balance normal. Ninety-six per cent absorption on an intake of 74.6 g per day.

Serum cholesterol (mean of 2 readings) 187 mg %.

Total serum lipoids 680 mg %.

Serum electrolytes normal.

Serum CO_2 (mean of 7 readings) 25.4 mEq.

Serum sodium (mean of 5 readings) 139 mEq. Range 128 to 142 mEq.

Serum potassium (mean of 5 readings) 4.1 mEq. Range 3.8 to 4.4 mEq.

Serum chlorides (mean of 5 readings) 106 mEq. Range 98 to 114 mEq.

Oedema fluid proteins, 1.4 g %.

Urinary aldosterone excretion (Courtesy of Dr. B. Singer, Dept. of Medicine, University of Sheffield) 3 micrograms per 24 hours (normal 0–5 mcg). (Neher & Wettstein, 1956.) Hess capillary fragility test—normal.

Capillary microscopy—normal.

Haemoglobin 13.6 g %.

Mantoux test positive (10 T.U. of O.T.).

X-ray of chest—persistent slight segmental collapse in the right lower lobe. A bronchogram did not show bronchiectasis.

Urine chromatography (Courtesy of Professor C. E. Dent, University College Hospital London)—no abnormality in the amino-acid and indole excretion.

Experimental Studies

The following additional investigations were carried out.

(1) Additon of potassium to the diet. Eight g of potassium citrate were added to his daily diet for 3 weeks, without effect on his oedema. There was an associated rise of serum potassium level from 4.1 to 4.6 mEq per litre.

(2) Sodium loading and elimination test. Ten g of sodium chloride were added to his daily diet. He excreted up to 7800 mg in 24 hours (270 mEq per litre). There was no change in his oedema. On a sodium free diet for 7 days he passed 680 to 1400 ml of urine per 24 hours. The total sodium output fell from 2140 mg per 24 hours (98 mEq per litre) to 1060 mg per 24 hours (62 mEq per litre). Throughout both tests the weight remained stationary, and the visible oedema remained unchanged.

(3) Water loading test. The whole load of one litre was excreted within 2 hours. The

Fig. 2. Pitting oedema of the hand.

Fig. 3. Pitting oedema of the foot with deformity of his toenails.

specific gravity fell from 1015 at the beginning of the test to 1001.

(4) Injection of 1 ml of Mersalyl. There was no diuresis or loss of weight.

(5) Hydroflumethiazide ("Naclex"), in a dose of 100 mg daily for 5 days, and then on alternate days for 72 days, along with potassium chloride 3 g daily. There was a slight diuresis lasting for a few days, but during the 2½ months he gained 900 g in weight.

(6) Elevation of the legs. When both legs were elevated to an angle of 45° for 90 minutes, and when only one was elevated for 3 hours, there was no difference in the oedema.

(7) Radioactive iodinated albumin studies (Taylor *et al.*, 1957) (Courtesy of Dr. J. D. S. Hammond, Department of Medicine, University of Sheffield), 2 ml (1.7 μ curie) were injected subcutaneously into the lower limbs. After 4 hours insignificant radioactivity had entered the blood stream, and even after 27 hours, during which time the patient had been normally active, so that lymphatic return was encouraged, the percentage of the injected amount which had reached the blood stream was only 30×10^{-4}. Dr. Hammond concluded that this signified a generalised dysfunction of the lymphatic system, and that this was probably the cause of the oedema.

Discussion

We showed that this boy's oedema was not due to cardiac diesease, liver disease, renal disease, hypoproteinaemia or abnormality in the serum proteins, aldosteronism or other electrolyte imbalance. It was not influenced by posture, diuretics, addition or removal of sodium, or addition of potassium. There was a positive nitrogen balance, a normal fat balance test, and a normal dietary intake. The oedema was of the pitting variety. It was not associated with any symptoms and caused no disability.

The explanation of the oedema was provided by the radioactive iodinated albumin test which indicated that there was an abnormality of the lymphatic system. The oedema, nevertheless, was of the pitting variety. The condition, in some respects is therefore similar to Milroy's disease, though the generalised distribution of the oedema and the complete absence of family history is against this diagnosis. We have not encountered any case in the world literature which was identical to the one we now report.

Summary

An otherwise healthy 12-year-old boy was investigated because he had generalised oedema since early infancy. Extensive studies failed to reveal renal, cardiac or metabolic disorders. There was no hypoproteinaemia and no steatorrhoea. Radioactive iodinated albumin injected subcutaneously did not reach the blood stream in adequate concentration even after 27 hours. This suggested that this was lymphatic oedema, but the cause of the lymphatic obstruction could not be determined.

Acknowledgments

Our thanks are due to Dr. J. D. S. Hammond and Dr. B. Singer, both of the Department of Medicine in the University, for the radioactive iodinated albumin test and for the aldosterone studies respectively: to Dr. J. L. Emery and Miss E. Finch of the Department of Pathology, the Children's Hospital, for the numerous investigations: to Professor C. E. Dent, of University College Hospital, London, for urine chromatography: to Professor D. A. K. Black, of the University of Manchester, Dr. T. Stapleton, of St. Mary's Hospital, London, and Miss W. Young, of the Queen Elizabeth Hospital for Children, London, for their helpful advice.

Addendum

After this paper had been completed Everley Jones (Symmetrical Peripheral Oedema in Infants. *Arch Dis Child, 35:* 192, 1960.) reported seven infants who had had widespread, persistent oedema of the extremetities, and five of these had symmetrical dystrophy of their toenails. It may well be, therefore, that the dystrophy of the toenails in our patient was an integral part of the clinical picture.

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CASE REPORT

Dermatitis Caused by Mites Living on Cats

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The literature records only few cases of dermatitis in man caused by mites living on cats (1).

Case: A.U.K., female, 11 years. Record No. 46 02 13. No family history of allergic manifestations. Transient itching for the last two years after eating apples, pears, peaches, nuts, and strong cheese. Urticaria once after sulphonamide medication for otitis associated with measles.

During the past six months scattered pale red papules slightly larger than a pin-head had occurred over the trunk and extremities. Some of these papules were found on examination to be scratched open and encrusted. Pigment patches after old lesions were present in places (Fig. 1). Itching was fairly troublesome and worse in the evenings. The girl had received no medication other than Lergigan for a short while during this period.

The general physical examination revealed nothing abnormal. The red and white blood picture and erythrocyte sedimentation rate were normal. No worm ova were found in the perianal region or in the faeces.

After 10 days' hospitalization with mild local therapy, the patient became free from symptoms. After as little as 24 hours at home the dermatitis recurred and she was readmitted three weeks later to the Department of Dermatology. The eruptions were then of the same type as earlier. We examined a cat she had at home and found in its fur a large amount of parasites which in appearance resembled Cheyletiella parasitivorax (Fig. 2). *We succeeded in demonstrating this mite in a papule on the girl's forearm.* The second period of hospitalization lasted for 10 days,

Fig. 1.

Fig. 2.

after which she was discharged healed. However, we kept the cat at the hospital and this time the dermatitis did not recur when the patient returned home, but it did follow upon the later return of the cat. After treating the cat with D.D.T. powder, the girl's dermatitis healed and did not recur again. Her parents were asymptomatic throughout.

Discussion

The repeated positive exposure tests and the demonstration of mites on the cat and in one of the patient's papules verified the diagnosis. The fact that the patient was the only one of a family of three to be affected might suggest allergic sensitization to the mite or its products, although the differing attraction of human beings to parasites is sufficient to account for this selectivity. The transfer of mites from one animal species to another is familiar, as is also the fact that the mites as a rule do not propagate in the new host. This is well illustrated in the present case by the spontaneous healing which occurred each time the child and the cat were separated.

Summary

A case of pruritic papular dermatitis in an 11-year-old girl is described. The cause of the eruptions was a mite found in the fur of the patient's cat and in one of the girl's papules. The dermatitis subsided twice after 10 days' hospitalization, only to recur immediately on her return home. The cat was later kept at the hospital and the patient was then free from symptoms at home. A new recurrence followed immediately on the cat's return. The cat was treated with D.D.T. powder. The patient has since been free from symptoms.

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Norwegian Pediatric Society

Meeting Jan. 29, 1960

Karl W. Wefring: Infantile Spasms

Infantile spasms are a form of epilepsy. The characteristic features are a series of rapid muscular contractions accompanied by cry, an invariably pathologic EEG, most frequently in the form of hypsarhythmia, and marked psychomotor regression. The prognosis has been very gloomy, the etiology unknown. Treatment with antiepileptic drugs has been of no avail. ACTH therapy has given more promising results, and where it is effective the spasms subside in the course of the first week of treatment, The treatment has to be instituted within two months of the onset of the disease if full mental remission is to be obtained.

Case: A.-C. J., girl, born February 1958. Previously healthy. At three months of age she had a febrile reaction following triple vaccine. Therefore, she was given only half the usual dose at four months of age. Two days later absence-like spells began and subsequently, daily flexor spasms associated with pronounced mental regression. The EEG revealed hypsarhythmia. In conjunction with measles in June 1959 the spasms subsided, the EEG was no longer pathologic by November 1959. The psychomotor development has improved and now, 23 months old, she has a developmental stage of a 12 month old.

Discussion. *Roald Rinvik:* Infantile spasms or salaam convulsions are a rare form of epilepsy. During 1959 we had 39 patients with convulsions at Ullevål Hospital practically all of whom were less than 2 years of age without once finding the EEG disturbances typical of infantile spasms. However, during the last few months we have observed two unquestionable cases. They have both been treated with cortisone. The two are infants, both born in June 1959. One of them had convulsions before, but not after admission to the hospital. The other had more uncertain symptoms prior to admission, but presented the typical convulsions after admission to the hospital. Both had the characteristic EEG changes. After 6 weeks' cortisone treatment both infants are free of convulsions. The EEG has, however, not improved.

There are many reports of good results of cortisone treatment in acute cerebral disorders. We used it successfully in a 10 month-old infant who developed generalized clonic convulsions after an episode of diarrhoea with severe dehydration. In this patient the EEG changes disappeared completely.

The well known phenomenon that epilepsy "disappears" during infections such as scarlatina etc. is probably not contingent on the infection per se. To induce hypothermia is considered by many to be appropriate therapy in certain cerebral disorders today; 15 years ago one used fever treatment. —*Kjell Aas:* It has been pointed out that ACTH can have an effect on acute pathologic conditions in the brain other than encephalitis. It is possible that cerebral edema brought on by an allergy, an autoimmunization process, may be the cause of infantile spasms. Seven children have been treated with ACTH in the Pediatric Department of the University Hospital. Five of the patients had the combination of infantile spasms and

hypsarhythmia; the other two had grand mal attacks in addition. One patient, who was microcephalic, reacted unfavourably to the treatment. Six patients had marked improvement clinically as well as by EEG. In one instance the recovery was of short duration and was followed by a complete relapse. The remaining 5 patients showed continued improvement, with increasing freedom from seizures, the EEG was normalized, and there was improvement of psychomotor behavior. However, the period of observation is short. One of the recovering patients had grand mal attacks mainly. The implication seems to be, however, that the extended use of ACTH or corticosteroids is indicated.

K. H. Torp: **Dysplasia of the Hip Joint in Tromsø**

Examination of 1,890 newborns over a period of 2 years in the Maternity Department, St. Elisabeth Hospital, Tromsø, has revealed the presence of Ortolani's sign associated with instability of the hip joint in a total of 15 cases. During the stay in the Maternity Department all infants were examined twice by the pediatrician. An independent examination was made by the obstetrician. In two infants the findings were questionable; in another two there was dissent between the two examining physicians. Frejka's cushion was, however, applied in all cases. Before discharge from the hospital the mother was made fully familiar with the background of the treatment and the technique. At 2–3 weeks of age the hip joints were clinically reexamined and adjustments of the cushion made when necessary. Roentgenographic examinations were performed at 4, 8, and 14 months of age. Of the 15 infants 13 were girls and 3 were breech presentations. One case of hip joint luxation occurred. Another infant, among the 1,875 who at the time of the examination had no suspicious signs, developed hip joint luxation later. The importance of individual evaluation and possibly even more extensive control measures than mentioned above in any newborn baby with suggestive signs of hip joint dysplasia is emphasized.

Discussion. *A. Njå:* Dr. Torp mentioned the possibility that the examination for Ortolani's sign might in itself injure the infant. I hardly think that is possible provided the examination is properly conducted. The examination should be made cautiously. Inexperienced physicians are apt to use too much force. In that case the characteristic findings may easily be overlooked and at the same time there is a risk of injuring the infant. The diagnosis of hip joint dysplasia will be missed occasionally in infants because of the absence of the sign in the newborn period. I know of 3 cases of hip joint luxation where I did not find a positive Ortolani's sign on examining the newborn infant. This occurred in a material of about 20,000 infants where dysplasia was demonstrated in about 100 cases. There is no reason to be wary of "overdiagnosing". There will always be many more dysplasias than the expected number of hip joint luxations in children. In some cases of dysplasia the first signs of a subluxation do not occur before puberty or during pregnancy, or in the later years of life as arthritis. All newborns with a positive Ortolani's sign should be treated even if the sign, as often happens, is no longer demonstrable after 1 or 2 weeks of age. — *Arne Kåss:* During the last 4 years about 5,600 newborns have been examined in Vestfold. Ortolani's sign was found positive in 9 infants (i.e. approximately 1/620). In no case has the examination caused injury. All infants have been treated with Frejka's cushion. The importance of keeping up the treatment long enough is stressed. In one patient treatment was gradually discontinued at 7 months of age when both clinical and roentgenographic findings seemed normal, a secondary luxation occurred. Treatment in the neonatal period was instituted without roentgenographic examination as it seemed of doubtful value. Recently a roentgenographic examination on the lines of the Swedish procedure described in Acta Radiol, February 1958 by Andrén-von Rosen has

been adopted and found helpful. So far, one case of hip joint dysplasia, not discovered in the routine examination of the newborns in this material, has been reported. — *M. Foss Hauge:* Dr. Torp's apparent uneasiness as regards the demonstration of Ortolani's sign so that no hip joint dysplasia be overlooked seems somewhat exaggerated; at least judging from the procedure he follows and his zeal for work. To demonstrate Ortolani's sign is mainly a mater of practice, and we, the orthopedic surgeons, obey the "Master's bidding" and *treat* these patients over a long period for hip joint dysplasia even though we do not always have proof that the sign be present. An increasing number of these patients are being referred to us at "Sophie's Minde Orthopedic Hospital" for treatment about one week after birth. However, if we send a note to the referring doctor stating that we have been unable to demonstrate Ortolani's sign or that the finding seems dubious this does not in the least imply a lack of confidence in that doctor's judgement. The reason may simply be that the symptom is no longer present, a thing which surely happens often enough; or it may be owing to lack of experience on our part. It is rather too early to present statistical material relating to the significance of an early diagnosis in hip joint dysplasia. But we have the impression that we no longer get so many children of one or two years of age with sub-or-total luxations for treatment as before. Apropos of Dr. Torp's remarks on roentgen diagnosis I would like to point out that in hip joint dysplasia it is made after careful evaluation of various factors: the caput epiphysis, the distance from and orientation towards the acetabulum and finally the anteversion. The latter must not be overlooked. It is a frequently occurring phenomenon and often the only symptom of a hip joint dysplasia.

Meeting March 11, 1960

Roald Rinvik: **Treatment of Diabetes in Children with Phenethyl-Formacidinyl-Iminourea**

In recent years a new group of antidiabetics, the biguanides, (sulfonyl carbamides), which are administered orally have come into use. These compounds cause blood sugar levels to fall in 90 per cent of diabetic patients. The mecahanism of action differs from insulin in that glucose liberation of the liver is reduced while muscle glycogen is increased. This leads to an increase of lactic acid in the surrounding tissues indicating a rise in anaerobic glycolysis, probably brought about by the inhibitory effect of the biguanides on certain oxidizing enzymes. The biguanides have no effect on the blood sugar of healthy persons. Digestive complications occur in at least one third of the cases in the form of anorexia, nausea and vomiting; no serious pathologic anatomic sequence has been observed. We have treated six children between 2 and 11 years of age, who have had diabetes for periods ranging from 3 months to 3 years, with phenethyl-formacidinyl-iminourea or D.B.I. The patients were treated with retard insulin and an adequate diet, maintaining a slight glycosuria. The diet remained unchanged throughout, and the effect of the treatment was judged from the patient's general condition and the possible reduction in the insulin doses. In three of the six patients the secondary effects were so severe the treatment had to be discontinued. In the remaining three cases no such complications occurred, but in two patients the therapy was of no avail as the insulin dose could not be reduced. In one case satisfactory improvement was obtained and the insulin dosage could be reduced from 16 retard units per day to 6 units.

However, in November 1959 there appeared a preliminary report (Walker) to the effect tht D.B.I. may cause acidosis and ketonuria even without the occurrence of hyperglycemia or glycosuria. Fatal cases

have been reported in this connection. The acidosis will probably be due to increased anaerobic glycolysis and a higher lactic acid level. In 4 of our 6 patients control of the alkali reserve was carried out revealing normal values. But with this element of danger peroral administration of D.B.I. can hardly be recommended in the treatment of diabetes in children.

Aagot Christie Løken, O. Hansen and S. Halvorsen: **Hereditary Nephropathy and Blindness**

Hereditary renal disease combined with other anomalies, such as nerve deafness and skeletal lesions has been reported recently. At the Pediatric Clinic, Rikshospitalet, Oslo we have had siblings (a boy and a girl) who died at 8 and 9 years of age in uremia. Both of them were blind and the boy was mentally retarded. From the age of 5–7 years they developed anorexia, vomiting, weight loss and a severe anemia resistant to any form of treatment. They had proteinuria, cylindruria, pyuria and isosthenuria. Eye examination showed reduced vision, the pupils reacted sluggishly to light and, in the girl, the eyegrounds showed a dusty pigmentation. Both died following a short period of sopor, vomiting and generalized seizures. An autopsy was performed on the girl. The kindneys showed infiltration of lymphocytes and plasma cells. Signs of renal dysplasia were present. The retina was aplastic with reduction of the outer granular layer. Renal dysplasia is supposed to reduce the resistance against infections and has been found to be a rather common cause of recurrent pyuria in children.

Both cases had two genetically determined anomalies, renal dysplasia and retinal aplasia. This combination of defects has not been reported previously. It is suggested that hereditary renal disease previously described associated with other anomalies and with different names, probably all have been cases of renal dysplasia combined with one or another anomaly.

Axel Støren: **Hypoglycemia**

Hypoglycemia caused by hypersensitivity to leucine is described in detail. The condition was first reported by Cochrane *et al.* in 1956. They found that in this condition leucine caused a hypoglycemic reaction even when present in blood concentrations no higher than normal. Hypersensitivity to insulin was also demonstrated, but the blood sugar response to adrenalin or glucagon stimulation was normal. Administration of adrenalin, glucagon, or glucose neutralized the hypoglycemia induced by leucine. The depressive effect of administration of glucose or insulin on the leucine level in serum in a normal person was further pointed out.

The case of an 18 month old boy, who was a patient in the Pediatric Department, University Hospital, Oslo, is reported: At about 8 months of age he developed symptoms of hypoglycemia during fasting or a few hours after meals. Low fasting blood sugar values (38 mg to 62 mg per 100 ml) were found. Insulin activity in plasma was normal or slightly elevated, making the diagnosis of hyperinsulinism unlikely. Adrenalin response was normal. The glucose tolerance curve was pathologic. There was a marked hypoglycemic reaction with convulsions after administration of 100 mg per kg dl-leucine. Examination for sensitivity to insulin was thought too dangerous. Treatment: A low protein diet (3.3 g per kg body wt) resulted in clinical improvement, but blood sugar values remained low. Prednisone was added in the dosage of 1 mg per kg. This resulted in marked improvement within a week. After that time the patient has remained free from symptoms on a daily prednisone dose of 0.3 mg per kg.

Hypoglycemia must be differentiated from epilepsy, and should always be born in mind as a possibility in children. Early diagnosis will prevent brain injury which otherwise frequently occurs.

Jens A. Steen: **Persistent Patency of the Omphalomesenteric Duct**

The case of a newborn boy with persistent patency of the omphalomesenteric duct is

reported. The patient was referred to the Pediatric Ward of Ullevål Hospital, because of erythroblastosis. A pea-sized red tumor was observed at the umbilicus, and after the cord had been cut a lumen from which meconium was aspirated was revealed. Roentgenographic examination with contrast disclosed direct connection with the small intestine. The patient is now 2 months old, and there has been only slight discharge from the umbilicus.

A review of 144 cases is reported in J Pediat 1952 by Scaletter *et al.* In 31 cases the intestine seems to have protruded through the open duct and only 4 of these survived. Sixty-seven other patients were operated upon with a fatal result in 8. Of the remaining 46 patients who received no treatment 12 died. The cause of death was not specified. It should, however, be noted that many of them occurred during the interwar period. The condition is also discussed by S. Cresson & G. Pilling in Pediat Clin N Amer, November 1959. They refer to the above mentioned publication and point out that surgical treatment should be undertaken as soon as the diagnosis has been made. In his "Surgery of Infancy and Childhood", 1956 edition, R. E. Gross likewise recommends surgical treatment. During the years 1940–50 he operated 11 patients all of whom lived. He does not mention when the operation should be performed.

We have been in some doubt as to when the operation should be performed in the present case. Since the radiographs show a fairly long duct and the possibility of prolapse of the intestine should be slight, operation at about 6 months of age will be recommended.

S. Halvorsen, Oslo

Acta Pædiatrica **49**: 759—762. Nov. 1960

BOOK REVIEWS

E. Rossi, E. Gautier & J. W. Weber: **Carbohydrate Metabolism in Children, Geigy Colloquium I, Bern, June 2–7, 1958. Edited.** Modern Problems in Pediatrics IV. S. Karger, Basle/New York 1959, 604 pp. Price Fr. 86 .– .

On the occasion of the 200 year jubilee of J.R. Geigy, Ltd, Basle, the University Children's Hospital in Berne organized a conference on carbohydrate metabolism with the collaboration of clinicians, biochemists and other specialists from 15 countries. The results represent a valuable review of our present knowledge in this field.

The first part of the book deals with *basic biochemical problems*. *C.F. Cori* discusses the differences between carbohydrate-metabolism in the liver and muscle, and the mode of action of insulin. A hypothesis of an intracellular spatial separation of different chemical operations is presented. The relationships between permeability, penetration and phosphorylation are demonstrated. *W. Wilbrandt* analyzes the kinetics of sugar transport, emphasizing the differences between sugar transport in the gut, kidney and red blood cells, where insulin has no effect and in the muscle cell, where glucose transport is under the control of insulin. — *J. Frei et al.* describe the results of a detailed micro-analytical study of enzyme systems in the liver and in leucocytes in cirrhosis, diabetes, and other pathological conditions. — *A.E. Renold et al.* report the results of systematic investigations of the metabolic activity of isolated adipose tissue and have been able to confirm the important role of adipose tissue in day to day regulation of energy metabolism, as demonstrated by the direct conversion in the fat tissue itself of glucose to fatty acids and the high metabolic activity of unesterified fatty acids (FFA) released from adipose tissue. The effects of various hormones, especially insulin, on this tissue was studied by means of an isotope technique, and a sensitive method for measuring insulin-like activity in biological fluids is elaborated. This method is however not yet suitable for clinical purposes.

Several papers discuss *glycogen storage disease*. *F. Linneweh* and *G. M. Guest et al.* stress the value of the dihydroxyacetone tolerance test when diagnosing this and other hepatic disturbances. As to the prognosis of glycogen storage disease the opinions are divergent: *R. Burgarelli* and *M. Jeune et al.* had observed late cardio-vasculo-renal complications, but according to *P. Royer* a striking improvement occurs at puberty.

An excellent review of *glucagon problems* is given by *P. P. Foa et al.* Glucagon, secreted by the alpha-cells of the pancreatic islets, is a potent, fast-acting hormone, which stimulates liver glycogenolysis and is an integral part of the blood sugar regulating mechanism.

U. Butturini, *E. Rossi* and *A. Linke* were able to show that glucagon increases peripheral glucose utilization, thus confirming the theory of Bürger that insulin and glucagon in synergy accelerate carbohydrate metabolism. Three different types of blood sugar response after glucagon administration to young diabetics were shown by *G. Hug*, indicating different degrees of endogenous insulin activity. — *W. Greutzfeldt* has demonstrated hydropic degeneration of the alpha-cells after treatment with biguanides (DBI) but did not consider these substances as primary alpha-cell poisons. The observed changes are probably an expression of a functional exhaustion secondary to disturbances in liver function.

P. Royer and *H. Lestradet* present a series of 86 cases of *periodic vomiting* and stress

the varying etiology of this condition, although in 23 cases the cause could not be established. It is emphasized that hypochloremic alkalosis with hypopotassemia is the rule during attacks, keto-acidosis being rare. It is also interesting that an increased excretion of 17-hydroxycorticoids occurs during the acute phase. *P. Royer* also reports 26 cases of *spontaneous hypoglycemia* and discusses different therapeutic possibilities according to etiology. The syndrome of familial hypoglycemia after leucine ingestion was discussed by *W. W. Payne* and *L. J. Woolf*. *R. Zetterström*, in two cases, found signs of hypofunction of the adrenal medulla, and continuous epinephrin treatment resulted in the patients becoming symptom-free. A low level of blood corticoids in the same disease was found by *R. Francois et al.* It is evident that there are many etiological factors in idiopathic hypoglycemia and the syndrome is no therapeutic entity.

Galactosemia is discussed in several papers. According to *A. Holzel* and *G. M. Guest* this is no rare disease and should always be suspected when glycosuria is found in an infant. The clinical symptoms are caused by the accumulation of galactose-l-phosphate in the tissue. The metabolic error is characterized by a practically complete inactivity of a specific "incorporation enzyme", galactose-l-phosphate uridyl transferase, as shown by *H. M. Kalchar* in studies on erythrocytes. It is interesting to note that they could demonstrate a partial defect of the same enzyme in the heterozygote parents of affected children. Characteristic electro-encephalographic changes are described by several authors.

An important contribution is made by *J. P. Febler et al.*, who in both acute and long-range tolerance tests compared the metabolism of glucose, fructose, galactose and sorbitol in normal and diabetic subjects. In diabetes, fructose administration was followed by an increase in blood glucose, which did not occur normally. This finding was considered to indicate increased gluconeogenetic activity of the diabetic liver. When fructose was given to a normal person under glucocorticoid treatment the same "diabetic" type of response was obtained. The increased gluconeogenesis was much more obvious in the juvenile type of diabetes than in the adult-onset-type.

F. Reubi analyzed the renal function in 14 cases of so-called *renal glycosuria*. They could be classified in two different types, one characterized by a low TmG, i.e. a low capacity of the tubules to reabsorb glucose, the other without such a defect. In the latter type an increased "dispersion of tubular activity" was believed to cause the disease, assuming that even under normal conditions functional differences exist between the nephrons.

The blood sugar level of *newborn children of diabetic mothers* has been studied by *N. Hallman*. When comparing the results of the Hagedorn-Jensen method with that of Somogyi–Nelson, which gives true glucose values, he found much greater differences in newborn infants than in older children. These differences were still greater in children of diabetic mothers. No explanation is given for these results. As regards the therapy of infants of diabetic mothers the conference agreed upon the following rules: a) the stomach should be emptied after delivery, b) the child should be put in an incubator with a high degree of humidity and a low oxygen concentration (30%), c) food or liquid should not be given during the first 36–48 hours, d) the child should be considered as a prematurely-born infant, carefully watched for cardiac or pulmonary distress but otherwise let alone as much as possible. In exceptional cases administration of glucose, potassium and/or digitalis may be necessary.

Diabetes mellitus was discussed in the last part of the conference. *H. Lestradet* could show that in diabetic patients without exogenous insulin the utilization of glucose at a high level of blood glucose was nearly the same as in non-diabetic subjects. He considered the hyperglycemia as "une adaptation utile", an extracellular glucose hypertension which promotes the penetration of

glucose into the cells. In agreement with other participants in the conference Lestradet added, however, that permanent hyperglycemia was not desirable and ought to be therapeutically controlled. An EEG study in diabetes was presented by *J. Chaptal et al.* Abnormal cerebral activities were only found in hypoglycemia. In the concluding discussion the incidence of angiopathy in 41 cases of longterm diabetes was demonstrated by *W. W. Payne.* It was the same as in other materials, i.e. about 80% retinopathy after more than 15 years of diabetes. It was emphasized that in the future when judging different types of treatment more attention must be given to the blood lipids than to the blood-sugar level.

Yngve A. A. Larsson

H. P. G. Seckel: **Bird-headed Dwarfs.**

S. Karger, Basel and New York 1960. Price SFr. 54.

The bird-headed or nanocephalic dwarf is characterized by an extremely short stature (minus 6 to 8 standard deviations), a small head, mental retardation and the frequent presence of congenital malformations including a beak-like nose. This very rare type of dwarfism is discussed in detail and from different points of view. The author advances the theory that in the nanocephalic dwarf the normal-size cells divide more slowly than in humans of normal stature, a process which begins at an early embryonal stage. A part of the monograph is a study in developmental anthropology and the book is of great value to those who are interested in dwarfism in general.

C. G. Bergstrand, Stockholm

The Royal Edinburgh Hospital for Sick Children.

Douglas Guthrie and Coll. E. & S. Livingstone Ltd. Edinburgh/London. 75 pages, numerous photos. Price 17/6 net.

A number of children's hospitals in Europe and the United States has now passed their 100th anniversary. In Great Britain the Hospital for Sick Children in Great Ormond Street was the first one to do so (1952), and now the turn has come to the Royal Edinburgh Hospital for Sick Children. In commemorating this event this fine jubilee book has been published. It gives the history of the Hospital, rather common to all similar institutions: a private hospital at first housed in a rented flat, then, with increasing need of beds, a separate building which in its turn has been replaced by new buildings with the increasing demand on the hospital; initially, and for a long time exclusively, run by money raised from private persons and private funds, and when these means no longer sufficed, supported by official sources. The Royal Edinburgh Hospital for Sick Children is now ready for a new rebuilding and modernization. The recent development and the future of the Hospital has found a chronicler in the professor of Child Life and Health, R. W. B. Ellis and an honourable tribute is paid to the leading personalities among the physicians, matrons and nurses, by some other members of the present staff. The Royal Edinburgh Hospital for Sick Children is to be congratulated on its achievements during its first 100 years and is offered best wishes for the future.

H. Schönenberg. **Der Liquor Cerebrospinalis im Kindesalter.**

Georg Thieme Verlag. Stuttgart. 1960. Price DM 29:—.

This is a volume of great value for pediatricians and clinicians, and of interest and help for those engaged in research concerning the cerebrospinal fuid. If one has to criticise it, the fact that the large survey of the literature contains only a small proportion of authors publishing in other languages than in German is unfortunate. The first part contains important data on the physiology of the cerebrospinal fluid with quite detailed descriptions of certain laboratory methods. In the later part the cerebrospinal fluid in different diseases is described very extensively. One can hardly find anything of value missing

in this book. This is so because the method of writing is rather concentrated. The 156 pages of facts, and bibliography of 14 pages are nicely printed with good photographs in black and white.

O. Wacz-Höckert, Helsingfors

***Nathan B. Talbot M. D., Robert H. Ritchie M. D., and John D. Crawford M. D.;* with charts by *Edith S. Tagrin:* Metabolic Homeostasis.**

Harvard University Press, Cambridge 1959: 132 pages.

Described by the authors as "a syllabus for those concerned with the care of patients", this work represents the further statement of a concept which has been developed over the recent years by the group of workers at the pediatric department of the Massachusetts General Hospital. Namely, that of a therapeutic approach, in different disease states, which aims at the restoration of, or compensation for, the body's disordered homeostatic balance. These workers have previousely expressed these ideas in published articles concerning rationales of fluid therapy and the management of renal disease.

The present work, which is expertly illustrated with graphs and diagrams, is divided into two sections, the first of which deals with the fundamental concepts of the normal balance of water and electrolytes. In the second section this material is translated into its clinical equivalent and applied with illustrative cases to the areas of infant feeding, parenteral fluid therapy, and renal disorders. In addition the authors propose the use of surface area rather than the hitherto more commonly used body weight as a frame of reference for calculating therapuetic doses in pediatric practice. To facilitate this an easily convertible graph is incorporated by means of which, body weight, surface area, and per cent of adult dose, are correlated.

The work is a rational approach towards therapy, and has a sound basis in the physiology of the human organism. It is recommended for all those interested in fluid and electrolyte balance, particularly persons concerned with the clinical care of children with pertinent disorders.

Leo Stern, Stockholm

***Kurt Hartung:* Strahlenbelastung und Strahlenschutz in der pädiatrischen Röntgendiagnostik.**

26 figs., 97 tables and 128 pages. Georg Thieme Verlag, Stuttgart, 1959. Price: 18: 60 DM

In the introductory chapters a broad survey of the different biological factors that are of importance in pediatric radiological work is given. These are followed by an extensive account of the results of an abundant series of measurements of skin doses and gonad doses under different conditions. In addition a survey of the effect of intentional variation of some physical factors of the rays is presented. A close screening of the measurements have, however, put some doubt in the reviewer's mind as to the applicability of the results and there are some statements that need correction. The meaning of the "effective value without Pb", which is fundamental for the evaluation of the results, has not been given with enough clarity. The sizes of the radiation fields and the thickness of the filters have not been defined. The suggestion to use a low KV as well as a low mA is hard to understand. In addition the main part of the examinations has been performed in the range of 50 to 60 KV, which does not seem to apply to prevalent conditions of current routine. Examinations, that in similarity to urethrocystography require a direct exposure of the gonads, are only scantily or not at all represented in the author's series.

The different suggestions of measures to increase radiation protection, given in the last section of the book are well-grounded.

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